THE MOLECULAR STRUCTURE OF EXUDATE GUMS, WITH SPECIAL REFERENCE TO GUMS OF THE COMBRETACEAE

by

JOHN J. CARLYLE, B.Sc.

A thesis presented for the degree of Doctor of Philosophy

University of Edinburgh

September, 1966

To my parents and Grace

C O N T E N T S

INTRODUCTION AND GENERAL CHEMISTRY OF THE PLANT GUMS

The word gum has for many years been used to designate a large number of naturally occurring related or unrelated substances, which give highly viscous aqueous solutions. In this thesis, however, it will be used exclusively to signify those carbohydrate materials which are exuded from the external parts of trees and fruits, spontaneously, after mechanical injury, or after attack by micro-organisms.

A surprisingly large number of plants exude gum. Whereas some trees only produce gum in small amounts, others, for example many species of Acacia, produce it with such profusion that it may be collected on a commercial basis. Gums have been used since civilization began. The Egyptians used them for paint thickening and embalming purposes as far back as 2000 B.C. and today gums serve a wide variety of purposes including sizing, the printing and finishing of textiles, paper making, pharmaceutical preparations and in the confectionery trade.

The gum is exuded from the tree in the form of a sticky mobile solution, which on exposure to the air dries to give hard glassy masses. Such gum nodules as they are called are composed almost entirely of polysaccharide material, and it is not uncommon for more than one different polysaccharide to be present. These polysaccharide components may each contain up to four main neutral sugar residues together with one or two

acidic sugar residues and are thus heteropolysaccharides. On account of the large number of different sugar residues which may be present and of the variety of ways in which these may be linked together, these natural polymers rank among the most complicated chemical systems known.

The formation of gum is stimulated by environmental conditions, mechanical injury to the bark, or by the attack of micro-organisms. Exudation at the injured site then provides a shield against loss of moisture or further attack.

The suggestion that gum exudates are true products of plant metabolism is supported by the fact that their underlying structures are often found to have some relationship to the botanical origin of the tree from which they were exuded, this being well demonstrated on consideration of the structures of gums from trees of the genus Acacia. Further evidence on this point is obtained from observed structural relationships between gums and other plant polysaccharides.

The polysaccharide material in the gum nodule exists as the salt of metals such as sodium, potassium, calcium or magnesium. In this respect the exudate gums differ from the pectic substances in which the carboxyl groups are generally methyl esterified. The polysaccharide, or gum acid, may be obtained by pouring an aqueous solution of the gum into an acidified organic solvent, generally ethanol. The polysaccharide is precipitated out of solution and further purification may be carried out by re-precipitation, dialysis or electrophoresis, after which it may be recovered by freeze-

drying, or by re-precipitation and subsequent drying by solvent exchange.

It is sometimes found that the gum nodule is insoluble in water. This is particularly so if the polysaccharide is partially acetylated and in this case de-acetylation with concomitant dissolution may be effected on treatment with alkali. The use of alkali must, however, be exercised with caution since, especially at elevated temperatures, degradation may occur[1,2,3].

The assessment of homogeneity of the starting material is an essential stage prior to the commencement of structural studies. Since no single unambiguous method is available, however, the complete homogeneity of a sample cannot be established directly, but on the other hand deviations from homogeneity may be established by examining the sample by as many methods as are available. Such an assessment is of imperative importance, since new chemical and biochemical techniques have been developed to determine the fine structure of polysaccharides. In the past many polysaccharides were considered to be homogeneous purely due to the inability to effect fractionation by the methods available.

The degrees of heterogeneity in plant gums may be regarded as extending from the micro-heterogeneity as exhibited in Combretum leonense gum to the gross heterogeneity as depicted by Khaya senegalensis gum.

In the case of Combretum leonense gum[4] while two components were shown present and separated by chromatography

on DEAE-cellulose, structural studies on these two polysaccharides by partial acid hydrolysis and methylation analysis gave qualitatively similar results. Furthermore ultracentrifugation of the two samples showed in each case a single peak having the same sedimentation coefficient. These results therefore provide evidence for the presence of two structurally different polysaccharides but suggest that these two components are composed of the same structural units linked in a similar manner but present in very slightly different proportions.

In contrast to the micro-heterogeneity as exhibited by Combretum leonense gum, the gum from Khaya senegalensis[5] has been shown to contain two polysaccharide components which structurally bear little resemblance to one another and are different even with respect to their component monosaccharides. One of these polysaccharides, the major component, is structurally similar to the gum from the related species, Khaya grandifolia[6] and to other galacturonorhamnans. On the other hand the minor component, having a main chain of 1→3 linked D-galactopyranose residues to which other D-galactopyranose residues linked through position 6 are attached as side chains, shows a marked resemblance to gums of the genus Acacia[7] and to other gums of the galactan family.

A second example of such gross heterogeneity is exhibited in the case of gum tragacanth[8,9] which has been shown to contain an arabinogalactan and a complex polysaccharide.

As an intermediate between these two cases of heterogeneity is the gum from Anogeissus leiocarpus[10]. While work on the

two components of this gum is not yet complete, studies so far suggest that while both contain the same monosaccharides and give the same oligosaccharides on partial hydrolysis, the relative amounts of these hydrolysis products in the two polysaccharides are quite different.

The number of techniques used on a preparative scale in the fractionation of polysaccharides from exudate gums, is limited by the relatively large amounts of material which are required for structural analysis. The methods which are normally employed fall into two classes.

The first of these is based on preferential solubility and involves the addition of a non-solvent, generally ethanol or acetone, to an aqueous solution of the gum, followed by the immediate removal of those precipitated components having different solubilities. The method is normally not totally selective but good results are obtained if a number of re-precipitations are carried out. This method was used successfully in the fractionation of Khaya senegalensis gum[5] and Olibanum gum[11]. The method may also be carried out in organic solvents as in the fractionation of derivatives of polysaccharides. By this method, methylated gum tragacanth was fractionated into an arabinogalactan, an acidic polysaccharide and a methyl glycoside[8].

The second class of fractionation technique depends on the ability of polysaccharides to form insoluble salts with detergent cations such as cetyl trimethyl ammonium[12] or cetyl trimethyl pyridinium[13]. These detergent cations combine with

acidic polysaccharides to form salts which have varying degrees of solubility depending on the acid content. Polysaccharides with high uronic acid content form insoluble salts whereas those with low acid content generally give water soluble salts. By this method Anogeissus leiocarpus gum has been fractionated into leiocarpan A and leiocarpan B, which, with respect to uronic acid content, are considerably different[10].

Whereas the above two classes of fractionation technique have proved particularly useful with respect to plant gums, a number of others, notably adsorption chromatography and gel filtration, are known. While these additional methods have as yet only really been applied to hemicelluloses and mucopolysaccharides, their success in these spheres may lead to an extension of their applicability into other fields, including that of the plant gums.

After fractionation the homogeneity of fractions obtained via one or other of the above procedures may be established by chromatography on diethylaminoethyl (DEAE)-cellulose[14], electrophoresis or ultracentrifugation[15].

The homogeneity of the polysaccharide having been established, preliminary experiments may then be carried out. These include physical measurements such as optical rotation and methoxyl content. The nature and proportions of the monosaccharide residues contained within the polysaccharide may be determined by total acid hydrolysis followed by separation of the monosaccharides by column chromatography[16]. The proportion of acidic sugars alone may be found by the determination

of uronic acid anhydride content[18]. Alternatively the relative estimation of monosaccharides may be carried out rapidly on the micro-scale by paper chromatography of the hydrolysate, in conjunction with some micro-analytical technique for quantitative sugar estimation such as the phenol sulphuric acid method[17].

Structural investigations tend to fall into three classes depending on whether they are based on methylation, partial hydrolysis or periodate oxidation.

Information as to the mode of linkage of each sugar unit and, in certain instances, on the ring structure of the sugars is obtained from methylation studies. Evidence as to the proportion of branched, unbranched and non-reducing end groups is also obtained by this procedure.

Methylation studies do not, however, give any indication of the sequence of sugar units. This is obtained by partial hydrolysis studies in which mineral acid of varying strength is used to determine the order and linkage of sugar units, by the isolation of oligosaccharides formed during the breakdown of the polysaccharide molecule. A particularly mild form of partial hydrolysis is possible when the polysaccharide contains uronic acid residues. Here it is often found that the acidity of a hot aqueous solution is sufficient to cleave labile peripheral residues. This process, known as autohydrolysis, produces a relatively stable degraded polymer, structural studies on which yield information concerning the core of the molecule. Furthermore, by an examination of the cleavage

products, information may be obtained concerning the periphery of the molecule.

The procedure of acetolysis is complementary to partial hydrolysis. Since certain sugar residues, notably rhamnopyranose residues, are very labile towards partial hydrolysis, oligosaccharides containing these sugars at the reducing end are unlikely to be obtained. Cleavage of acetylated gum arabic[19], however, using a mixture of acetic anhydride and sulphuric acid as aceteolytic agent, was found to give oligosaccharides in which the L-rhamnopyranosyl bond was retained (see Fig. III). Similarly 2-O-α-L-fucopyranosyl-D-xylose has been isolated from gum tragacanth[9] and so acetolysis provides a valuable alternative method for the linkage analysis of polysaccharides containing 6-deoxyhexose residues, which are normally labile towards partial hydrolysis.

Cleavage techniques such as those outlined above are dependent on the fact that certain linkages within the molecule are more labile than others. Recently attempts have been made to modify the polysaccharide in such a way that subsequent partial fragmentation will yield an entirely different spectrum of oligosaccharides.

The first of these modifications involves the oxidation of primary alcohols to uronic acids, so rendering stability to otherwise labile linkages. For this method to be of maximum value the polysaccharide to be oxidised must have a minimum variety of sugar residues containing primary hydroxyl groups, e.g. in arabinoxylans[20] or in hexose containing polysaccharides

with a large proportion of 1,6 linkages.

The second method is practically the converse of the first and involves the reduction of uronic acid residues to the corresponding alcohols. By this method previously stable residues attain normal stability and so are much more easily cleaved. The application of this method is demonstrated with reference to the acetolysis of carboxyl reduced gum arabic (see Fig. III).

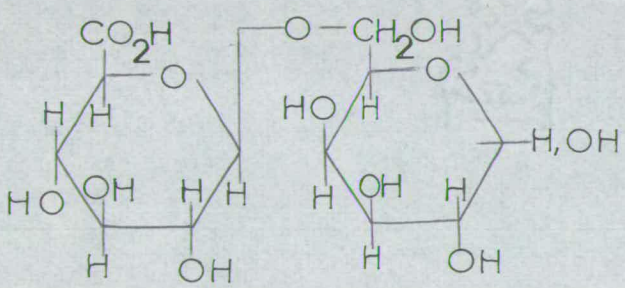
Periodate oxidation of polysaccharides is dependent on the fact that certain sugars are so substituted that they do not possess adjacent hydroxyl groups and are thus resistant to attack by the periodate ion. The application of this reaction coupled with reduction and hydrolysis[21] makes possible a new type of molecular cleavage. The nature of the partial hydrolysis products is dependent on the mode of substitution and in many cases the periodate resistant residues are so located that by this process, known as the Smith degradation, it is possible to obtain a degraded gum. The method may be exemplified by reference to gum arabic where it was possible to obtain a degraded gum consisting only of galactose residues which were mainly, if not exclusively, $1 \rightarrow 3$ linked[22] (see Fig. II).

Figures I, II and III demonstrate the application of some of the above techniques with respect to structural studies on gum arabic.

Figure I

Arabic acid —total hydrolysis(23)→ D-galactose (3.7)
L-arabinose (3.0)
L-rhamnose (1.1)
D-glucuronic acid (1.2)

Arabic acid —partial acid hydrolysis(24)→ D-GpA 1 —β→ 6 D-Gal...(a)
+ Monosaccharides



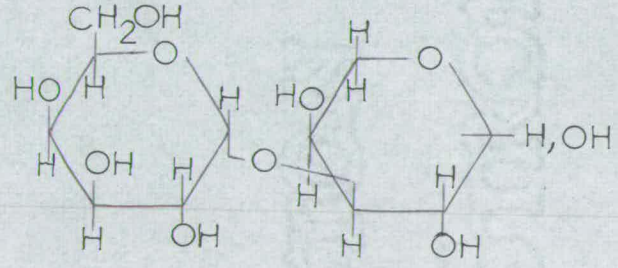
.....(a)

Figure II

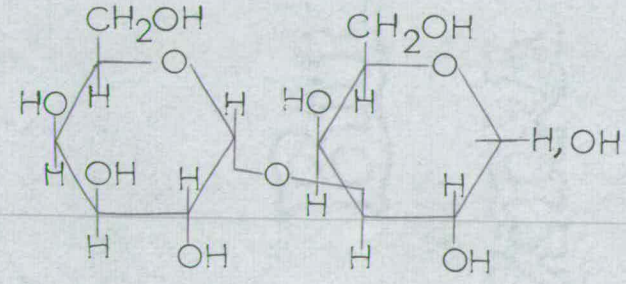
Arabic acid $\xrightarrow[34\ hr.,\ 100^{o}]{\text{Autohydrolysis}^{(25)}}$ L-arabinose
L-rhamnose
D-Galp 1 $\xrightarrow{\alpha}$ 3 L-Ara...(b)
+ Degraded gum A

Degraded gum A $\xrightarrow{\text{Further autohydrolysis}^{(26)}}$ D-Galp 1 $\xrightarrow{\beta}$ 3 D-Gal...(c)
+ Degraded gum B

Degraded gum A $\xrightarrow[\text{(iii) } \underline{N}\text{-}H_2SO_4]{\text{(i) } IO_4^- \text{ oxidation}^{(22)}\ \text{(ii) } KBH_4}$ 1⟶3 linked galactan



.....(b)



.....(c)

Figure III

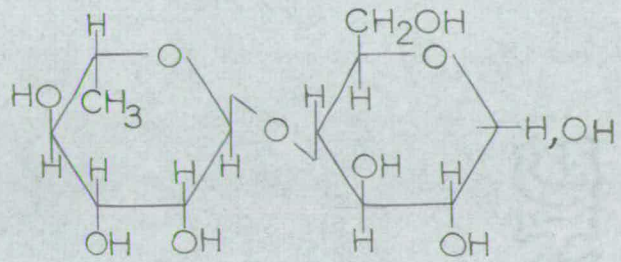
Arabic acid —[(i) acetylation[19] (ii) diborane reduction (iii) Ac_2O/H_2SO_4]→

L-Rhap 1 —α→ 4 D-G .....(d)

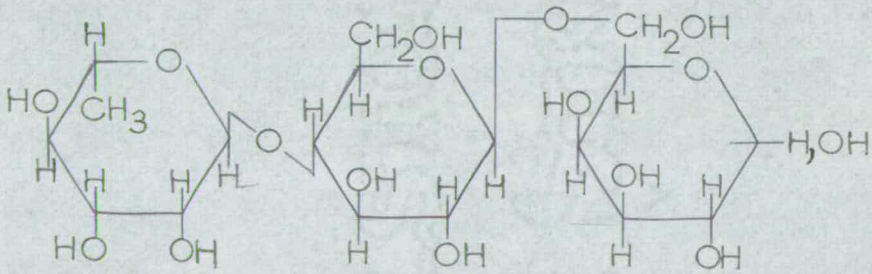
D-Galp 1 —β→ 3 D-Gal .....(c)

L-Rhap 1 → 4 D-Gp 1 → 6 D-Gal...(e)

+ other oligosaccharides



...(d)



...(e)

THE CLASSIFICATION OF PLANT POLYSACCHARIDES

The plant polysaccharides have often been referred to as hemicelluloses, gums, pectic substances and food reserves. From a chemical point of view, however, such terms are rather imprecise and with increased structural knowledge of some of these polysaccharides, it soon became obvious that such a classification was both inadequate and unsatisfactory.

The most recent classification of the plant polysaccharides is based on an understanding of their underlying basic structure[27]. It is often the case that the presence of similar arrangements of sugar residues within the main chain is adequate justification for the inclusion within the same group of polysaccharides, which may among themselves differ quite markedly with respect to other structural features, notably the location of other sugar residues in the periphery of the molecule. Thus the xylan, glucomannan and arabinogalactan types form three well-known familes of structurally related polysaccharides.

The classification of many of the more complex polysaccharides must be regarded as highly tentative, since although a number of oligosaccharides have been characterised as partial hydrolysis products, it is not yet known with certainty which of these are part of the main chain and which arise from outer chains.

The classification of these polysaccharides is in terms of basal chains of sugar residues and Table I gives some indication

of the groups into which certain plant heteropolysaccharides are placed.

Present in main chain	Represented by	Exemplified by	Ref.
Xyl 1→4 Xyl	arabinoxylans	rye flour arabinoxylan	28
	(4-O-methyl)-glucuronoxylans	wood hemicelluloses	
Man 1→4 Man	galactomannans	soy-bean galactomannan	29
	galactoglucomannans	Norway spruce galactoglucomannan	30
Gal 1→3 Gal and Gal 1→6 Gal	arabinogalactans	coniferous woods, e.g. Japanese larch	31
	complex polysaccharides	*Acacia* gums	7
Gal 1→6 Gal	complex polysaccharides	*Cholla* gum	32
GalA 1→4 GalA	galacturonans	pectic substances, e.g. Sunflower pectin	82
	complex polysaccharides	gums of *Astralgus*, *Khaya* and *Sterculia* genera	9 33 34

On account of the possible variations both in the nature of the sugar units and also in the diversity of ways in which these sugar units may be combined, the exudate gums represent the chemically most complex group of plant polysaccharides.

In spite of this structural complexity, the plant gums have structural features in common with other classes of plant polysaccharides as shown (Table I).

Those gums having an underlying framework of 1⟶3 and 1⟶6 linked D-galactopyranose residues to which arabinose residues may be attached, show similarity to the arabinogalactans which are normally considered to be hemicelluloses. Thus it is obvious that various Acacia gums, notably A. senegal[7] and A. pycnantha[35] and the gum from Araucaria bidwilli[36], belong to the same structural family of polysaccharides as do the arabinogalactans from the woods of conifers, e.g. larches[37-41] and Scots pine[42]. Furthermore, due to the similarity between the structures of those gums and those hemicelluloses outlined above, it is not unreasonable to believe that these arabinogalactans may be the precursors of certain plant gums and that the later stages of biosynthesis might involve the apposition of D-glucuronic acid, L-rhamnose and considerably more L-arabinose. This theory is supported by the fact that the arabinogalactans from tamarack larch[43], mountain larch[44] and maritime pine[45] have all been found to contain small amounts of D-glucuronic acid thus showing their structure to be intermediate in the structural spectrum, going from the true arabinogalactans to the exudate gums.

A further possible resemblance is that between the pectic acids and the complex exudate gums obtained from the genera Astralgus, Khaya and Sterculia. While pectic acid is known to be composed substantially of linear chains of 1⟶4 linked

D-galacturonic acid[46] it has recently become clear that pure galacturonans containing only galacturonic acid are in fact rare, and most frequently this acid appears in connection with residues of L-arabinose, D-galactose and L-rhamnose. There is as yet little evidence concerning the structural role of these sugar units but due to the isolation of 2-O-(α-D-galacto-pyranosyluronic acid)-L-rhamnose and higher oligosaccharides containing only residues of galacturonic acid and rhamnose from lucerne[83] and soybean hulls[47] pectic acids and in citrus pectin[48], it now appears that these oligosaccharides originate from the main chain of the pectic acid. The above aldobiouronic acid has also been shown to be present in Khaya gradifolia[6], Khaya senegalensis[33] and Cochlospermum gossypium[34] gums and in various Sterculia gums[34,49]. In these gums the L-rhamnose residue is interposed between blocks of 1→4 linked α-D-galacturonic acid residues. It is thus probable that such a structural feature may be common to both these plant gums and also to the pectic substances and if so the relationship between the galacturonorhamnan type plant gums and the pectic substances will be enforced.

THE CLASSIFICATION OF EXUDATE GUMS

The classification of the exudate gums involves a further development of those principles adopted in the classification of other plant polysaccharides. Due to the very great complexity of most plant gums, however, the difficulty of deciding those structural units which comprise the main chain is much more pronounced. As a further consequence of this complexity very few exudate gums have been characterised fully enough to be included in any classification. Nevertheless, on the basis of present evidence three quite distinct families have so far been recognized. These are as follows.

1. The galactan family

This family may be subdivided into (1a) those gums having a main chain of 1→3 linked β-D-galactopyranose residues, (1b) those gums having a main chain of 1→6 linked β-D-galactopyranose residues.

Sub-family 1a

Represented by the Acacia gums, A. senegal[7], A. pycnantha[35], A. mearnsii[50], and certain other gums notably Araucaria bidwilli[36] and Asafoetida gum[51], all the gums of this group have a main chain which is composed mainly if not exclusively of 1→3 linked D-galactopyranose residues to which are attached as side chains, other D-galactose residues joined by 1→6 linkages. In all these gums D-glucuronic acid (or its 4-methyl ether) is attached at the

ends of the galactose side chains, and from methylation analysis it appears that in Araucaria bidwilli and Asafoetida gums the acidic residues are attached exclusively as end groups. This is thus in contrast to what was found in Acacia senegal gum[19] where it was shown that some at least of rhamnose residues were attached to position 4 of the glucuronic acid.

Sub-family 1b

This group is represented by cholla gum[32] and, while a number of other gums, notably Combretum leonense[4], gum jeol[52], the gum from Virgilia oroboides[53-57] and apricot gum[58], have been shown to contain chains of 1→6 linked β-D-galactopyranose residues, there is, with these additional gums, as yet no definite evidence to suggest whether or not such structural units comprise the main chain of the molecule.

The Anogeissus gums, namely those from Anogeissus latifolia[59-62] and Anogeissus leiocarpus[63], do contain chains of 1→6 linked β-D-galactopyranose residues but it has now been established that in leiocarpan A[64] and gum ghatti (A. latifolia) such chains do not form the main chain of the molecule, and this may also be the case in leiocarpan B. These Anogeissus gums will be discussed later as members of the glucuronomannan family.

2. The galacturonorhamnan family

This family of plant gums are characterised by the fact that their main chain is composed of blocks of 1→4 linked α-D-galacturonic acid residues, and the group as a whole may

be regarded as showing a spectrum of structural types of polysaccharides going from tragacanthic acid[9], whose main chain is almost pure galacturonan, to those gums in which the blocks of galacturonic acid residues are interposed with rhamnose residues. Amongst gums of the latter type two distinct subclasses are recognizable (namely gums of the Sterculia and Khaya genera). Both of these contain glucuronic acid (or its 4-methyl ether) in addition to galacturonic acid. While the galacturonic acid is present mainly if not exclusively in the main chain, however, the glucuronic acid occurs exclusively as non-reducing end groups. In the first of these sub-classes, represented by gums of the Sterculia genus[34,49], the glucuronic acid is attached directly to the galacturonic acid of the main chain (Fig. IV), but in the second sub-class, represented by gums of the genus Khaya[6,33], the glucuronic acid (generally as its 4-methyl ether) is attached through galactose to rhamnose situated in the main galacturonorhamnan main chain (Fig. V).

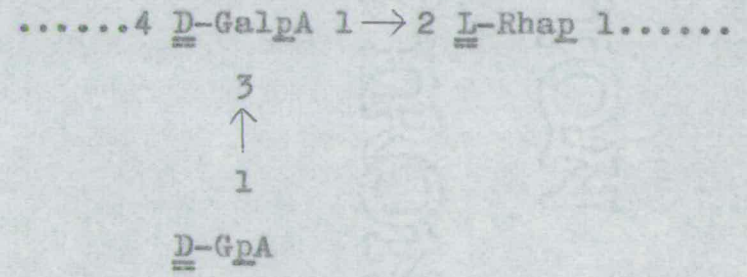


Figure IV

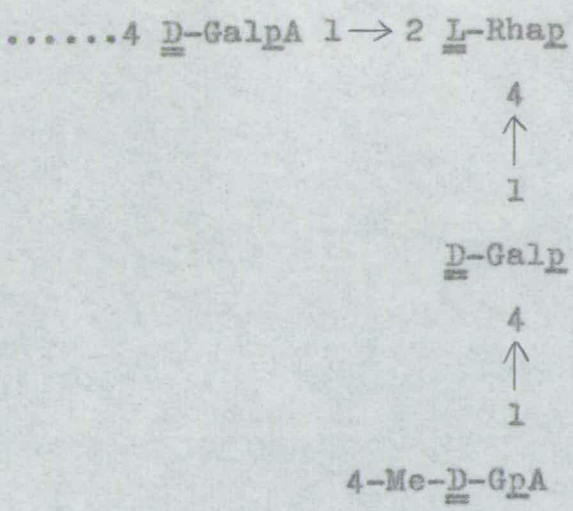


Figure V

3. The glucuronomannan family

This group of polysaccharides has so far only one fully characterised representative, namely leiocarpan A, in which the main chain has been shown to have an alternating sequence of D-glucuronic acid and D-mannose residues[64]. In a number of other gums, notably gum ghatti[59-62] and leiocarpan B, the aldobiouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose has been obtained as a partial hydrolysis product. In the case of gum ghatti, while it has been established that the above aldobiouronic acid arises from the main chain, the alternating sequence of D-glucuronic acid and D-mannose residues has still to be established, although recent results, to be discussed later, have shown that a tetrasaccharide containing such an alternating sequence is formed on partial hydrolysis.

It is thus probable that these gums also belong to the glucuronomannan family.

The chemistry of these gums along with the gum from the related species Combretum leonense is reviewed below.

THE CHEMISTRY OF GUMS OF THE COMBRETACEAE

The gums whose chemistry will be reviewed here are those from *Anogeissus latifolia*, *Anogeissus leiocarpus* (formerly *A. schimperi*) and *Combretum leonense*.

The gums come from botanically related species and show structural similarity to one another, especially with respect to the oligosaccharides formed on partial hydrolysis (Table II).

Table II

Gum	Periphery	Galactan framework	Acidic fragments
A. latifolia		Galp 1→6 Gal Galp 1→6 Galp 1→6 Gal Galp 1→3 Gal Galp 1→3 Ara Galp 1→6 Galp 1→3 Ara	GpA 1→2 Man GpA 1→6 Gal
A. leiocarpus	Araf 1→3 Ara Arap 1→3 Ara	Galp 1→6 Gal Galp 1→6 Galp 1→6 Gal Galp 1→3 Gal Galp 1→3 Ara Galp 1→6 Galp 1→3 Ara	GpA 1→2 Man GpA 1→6 Gal
C. leonense	Arap 1→3 Ara Galp 1→3 Ara	Galp 1→6 Gal Galp 1→6 Galp 1→6 Gal Galp 1→4 Gal	GpA 1→6 Gal GalpA 1→2 Rha

Anogeissus latifolia gum

Interest in gums of the Combretaceae was initiated by research on gum ghatti (A. latifolia). From extensive structural investigations carried out in this laboratory[59-62] many of the structural problems have now been solved and partial structures have been put forward.

On hydrolysis the gum was found to give L-arabinose (5), D-galactose (3), D-mannose (1), D-glucuronic acid (1), and D-xylose (0.5) in the approximate molar proportions shown. There was also a trace of rhamnose. Graded hydrolysis carried out on both the whole gum and the degraded gum B (Fig. VII) gave a wide spectrum of both neutral and acidic oligosaccharides (Fig. VII and Table III). The structural investigations carried out may be represented as below (Figs. VI and VII).

Figure VI

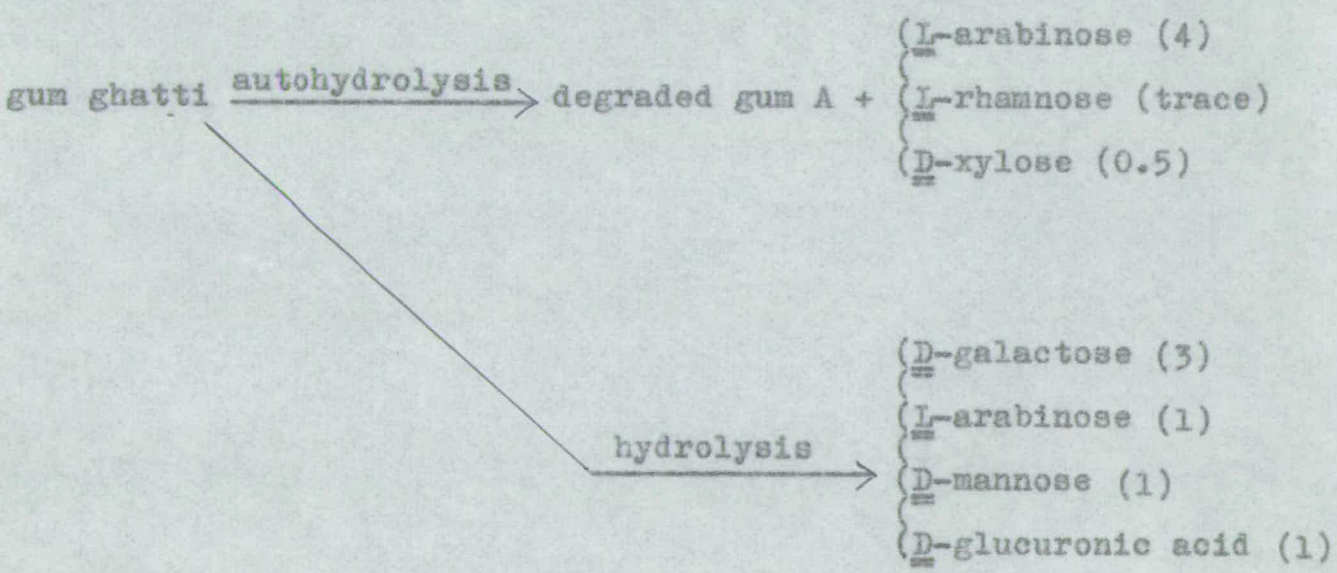


Figure VII

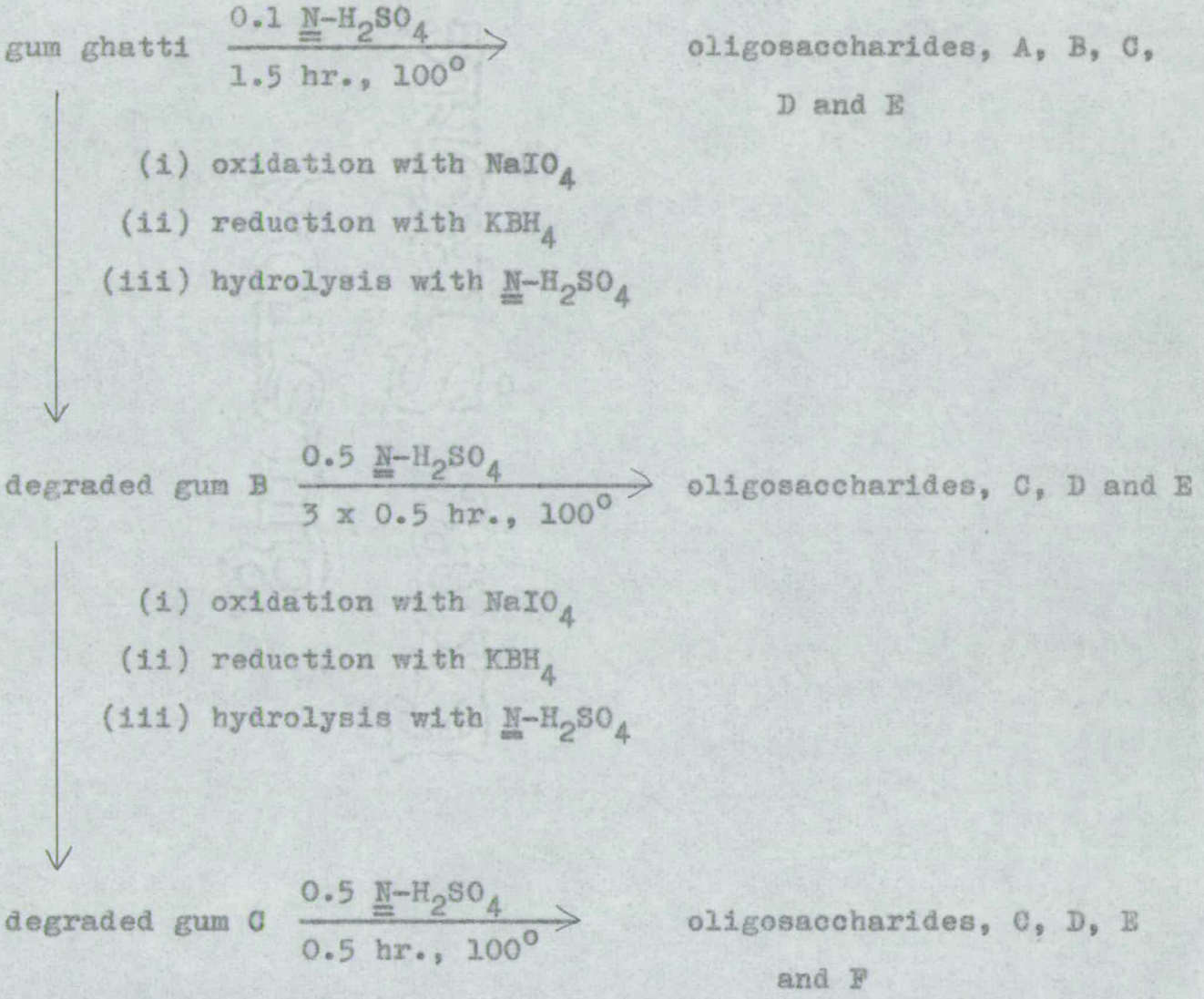
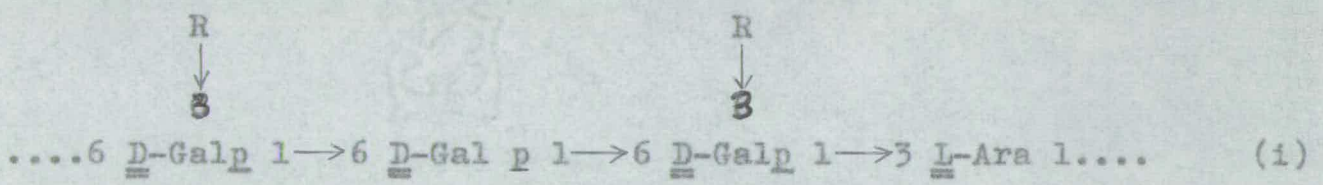

gum ghatti
0.1 N-H2SO4
1.5 hr., 100°
oligosaccharides, A, B, C, D and E
(i) oxidation with NaIO4
(ii) reduction with KBH4
(iii) hydrolysis with N-H2SO4
degraded gum B
0.5 N-H2SO4
3 x 0.5 hr., 100°
oligosaccharides, C, D and E
(i) oxidation with NaIO4
(ii) reduction with KBH4
(iii) hydrolysis with N-H2SO4
degraded gum C
0.5 N-H2SO4
0.5 hr., 100°
oligosaccharides, C, D, E and F

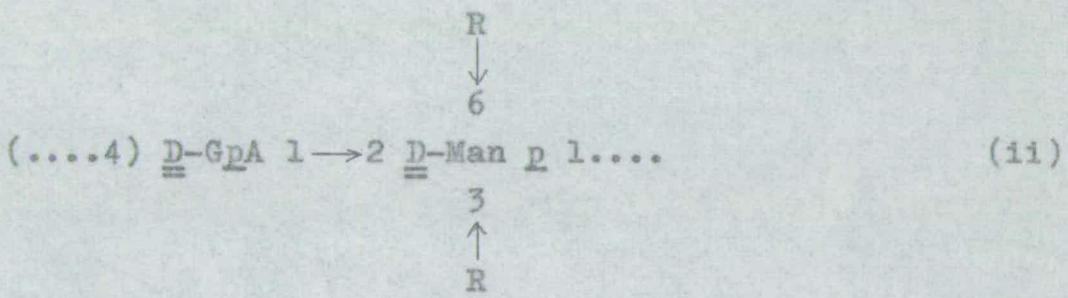
Table III

Oligosaccharide	Whole gum	Degraded gum B	Degraded gum C
A. D-GpA 1 $\xrightarrow{\beta}$ 6 D-Gal	+		
B. D-GpA 1 $\xrightarrow{\beta}$ 2 D-Man	+		
C. D-Galp 1 $\xrightarrow{\beta}$ [6 D-Galp 1]$_n$ $\xrightarrow{\beta}$ 6 D-Gal	+, n = 0-2	+, n = 0-4	+, n = 0
D. D-Galp 1 $\xrightarrow{\beta}$ [6 D-Galp 1]$_n$ $\xrightarrow{\beta}$ 3 L-Ara	+, n = 0-3	+, n = 0-3	+, n = 0
E. D-Galp 1 $\xrightarrow{\beta}$ 3 D-Gal	+	+	+
F. L-Arap 1 $\longrightarrow$ 3 D-Man			+

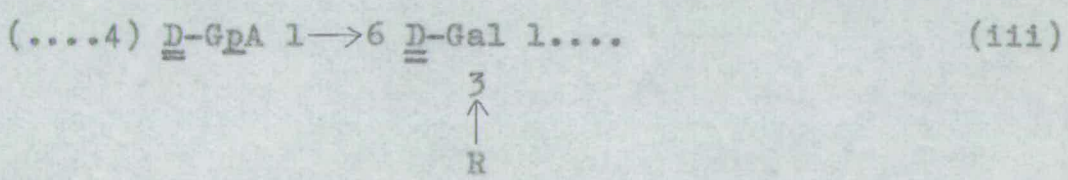
From these results coupled with the results of methylation analysis the following partial structures were put forward.



(i)



(ii)



(iii)

Autohydrolysis of the gum (Fig. VI) was found to remove 80% of the arabinose residues before the liberation of galactose was detected. In addition this process was found to eliminate all xylose and rhamnose, leaving degraded gum A, containing 4% of arabinose.

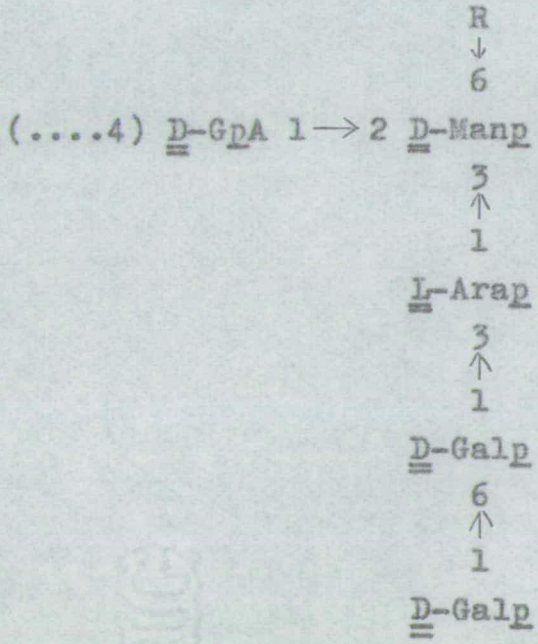
Degradation of the gum by Smith's procedure[62] gave degraded gum B (Fig. VII) which was examined by methylation and partial hydrolysis. Further Smith degradation of degraded gum B gave degraded gum C, which on partial hydrolysis gave a number of oligosaccharides amongst which the following three were characterised:

3-O-L-arabinopyranosyl-D-mannose

3-O-β-D-galactosyl-L-arabinose

6-O-β-D-galactosyl-D-galactose

These three oligosaccharides may be accommodated in partial structure (iv) which, assuming that the L-arabinose in (i) is in the pyranose form, provides the key between structures (i) and (ii).



(iv)

While the isolation of an aldotriouronic acid containing contiguous mannose residues was reported previously[61], in the light of recent evidence it now appears that this component is in fact a tetrasaccharide containing alternative glucuronic acid and mannose residues. This work will be discussed later.

2. Combretum leonense gum

Although Combretum leonense gum and the Anogeissus gums are obtained from species within the same botanical family (Combretaceae), C. leonense shows marked differences from the other gums, particularly with respect to the monosaccharides produced on total hydrolysis[4]. Furthermore in this gum the mannose containing aldobiouronic acid is replaced by 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose (Table II).

Nevertheless in spite of these obvious differences, Combretum leonense gum gives a similar group of oligosaccharides on partial hydrolysis (Table II). This gum has also been shown to contain chains of 1⟶6 linked β-D-galactopyranose residues, and so as in gum ghatti this structural feature appears to be an important part of the molecular architecture.

In leiocarpan A (see below) the 2-O-(β-D-glucopyranosyluronic acid)-D-mannose was shown to be located in the main chain. Although there is as yet no direct evidence to suggest that 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose is similarly located in Combretum leonense gum the possibility exists, and if this were in fact the case, then the association of this gum with the Anogeissus gums would be very much stronger.

3. Anogeissus leiocarpus (formerly A. schimperi) gum

Initial work on Anogeissus leiocarpus gum[64] showed that it contained the same monosaccharides, and on partial hydrolysis gave the same neutral oligosaccharides as did gum ghatti (Table II).

The two gums gave the same two aldobiouronic acids (Table II) but, whereas it was found that gum ghatti gave these in approximately equal proportions, from A. leiocarpus gum only the mannose containing oligosaccharide was formed in substantial amount.

Furthermore, as was the case in gum ghatti, A. leiocarpus was found to contain some arabinose residues in the interior chains of the polysaccharide since partial hydrolysis afforded the same homologous series of oligosaccharides, namely, O-β-D-galactopyranosyl-$\left[(1\rightarrow6)$-O-β-D-galactopyranosyl$\right]_n$-(1→3)-L-arabinose. The isolation of the same two series of oligosaccharides containing 1→6 linked β-D-galactopyranose residues from both gums demonstrated that the interior chains were similarly constituted.

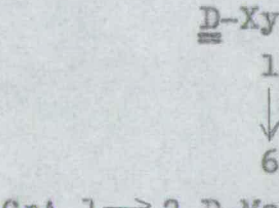
Recent chromatographic examination of Anogeissus leiocarpus gum on DEAE-cellulose[65] has shown that the gum is heterogeneous and by salt formation with cetyl trimethyl ammonium bromide may be fractionated into two polysaccharides, leiocarpan A (uronic acid anhydride content, ca. 30%) and leiocarpan B (uronic acid anhydride content, ca. 12%). Even more recently[10] structural investigations have been carried out on leiocarpan A, and these are summarized below.

Autohydrolysis of the gum designed so as to give a preferential release of arabinose, gave degraded leiocarpan A which was converted into its fully methylated derivative. Reduction of this methylated polysaccharide, followed by hydrolysis, gave a large number of methylated neutral sugars, the more abundant of which are listed below (Table IV).

Table IV

Methylated monosaccharide	Relative amount
2,3-di-O-methyl-D-glucose	++++
3,4-di-O-methyl-D-mannose	+++
3,4,6-tri-O-methyl-D-mannose	+++
2,3,4-tri-O-methyl-D-xylose	++
2,3,4-tri-O-methyl-D-galactose	+

The bulk of the components listed above (Table IV) may be accommodated in partial structure (a) which although agreeing with the results of methylation analysis is only one of the many partial structures for degraded leiocarpan A which may be advanced at this point.



....4 D-GpA 1→2 D-Manp 1→4 D-GpA 1→2 D-Manp 1.... (a)

A partial acid hydrolysis of leiocarpan A controlled so as to give a maximum yield of acidic oligosaccharides was carried

out. After fractionation of the hydrolysate into the individual sugars, evidence was obtained for the following (Table V).

Table V

Acidic oligosaccharides from leiocarpan A
β-D-GpA 1→2 D-Man
D-GpA 1→2 D-Manp 1→4 D-GpA 1→2 D-Man
β-D-GpA 1→6 D-Gal
D-GpA 1→6 D-Gal 1→3 L-Ara

Carboxyl reduced leiocarpan A was prepared by the treatment of acetylated leiocarpan A with diborane. Acetolysis of reduced acetylated leiocarpan A followed by de-acetylation of the products gave a wide spectrum of oligosaccharides containing residues of D-glucose and D-mannose (Table VI).

Table VI

Acetolysis products from carboxyl reduced leiocarpan A
β-D-Gp 1→2 D-Man
α-D-Manp 1→4 D-Gp
Gp 1→2 Manp 1→4 Gp
Manp 1→4 Gp 1→2 Man
Gp 1→2 Manp 1→4 Gp 1→2 Manp
Manp 1→4 Gp 1→2 Manp 1→4 Gp

The isolation of these oligosaccharides (Tables V and VI) shows that the main chain of leiocarpan A is composed of 4-O-substituted β-D-glucuronic acid residues and 2-O-substituted α-D-mannopyranose residues. Furthermore the absence of oligosaccharides containing contiguous residues of the same sugar shows that the two sugar residues are present in a regularly alternating sequence as in partial structure (a), and so the results from partial hydrolysis and acetolysis are in agreement with those from the methylation analysis of the degraded gum.

OBJECT OF THE PRESENT INVESTIGATIONS

Part I : Gums of the Combretaceae

Leiocarpan A

From previous work[10] it was established that leiocarpan A has a main chain of strictly alternating D-glucuronic acid and D-mannopyranose residues. In addition, the D-xylopyranose units were shown to exist mainly if not exclusively as non-reducing end groups attached directly to the main chain, through the 6 position of the mannose residues. The primary object of the present investigations has been further examination of leiocarpan A, particularly with reference to the structure of the side chains.

The pattern of substitution in the gum has been indicated after methylation analysis, and by the isolation of oligosaccharides from controlled acid hydrolysis, evidence has been obtained concerning both the order of sugar residues, and in certain cases the configuration of the glycosidic linkages. An acidic oligosaccharide which was isolated after graded hydrolysis of leiocarpan A[10] and was tentatively identified as

D-GpA 1 → 2 D-Manp 1 → 4 D-GpA 1 → 2 D-Man

has now been fully characterised. In conjunction with C.C. Whitehead, the same tetrasaccharide, but from gum ghatti, has been isolated and characterised.

In the light of results from the foregoing experiments further information on the structure of leiocarpan A was sought by the degradation of the carboxyl-reduced polysaccharide by

Smith's procedure. By this method evidence was obtained concerning the nature of sugar residues and mode of substitution at the junction of the main and side chains.

Leiocarpan B

Parallel experiments have been carried out on leiocarpan B. Due to the relative difficulty in obtaining substantial amounts of leiocarpan B, however, these experiments have had to be more incomplete than those on leiocarpan A. In spite of this, however, enough definite results have been obtained to enable a structural comparison between leiocarpan A and leiocarpan B to be made.

Part II

Acacia mearnsii gum

Preliminary experiments[50] have shown that this gum shows similarities to other gums of the *Acacia* genus, notably those from *A. senegal* and *A. pycnantha*. Additional experiments have now been carried out on *A. mearnsii* gum, and the results of these have given a firmer basis on which structural comparison with the other *Acacia* gums may be made.

P A R T I

THE ANOGEISSUS GUMS

DISCUSSION

DISCUSSION

The sample of *Anogeissus leiocarpus* gum used was the same as had been investigated by Aspinall and McNab[64]. The gum was botanically authenticated and had been obtained from the Tropical Products Institute. Previous investigations on the gum[10] established the presence of two discrete polysaccharide components, leiocarpan A and leiocarpan B, which were markedly different with respect to uronic acid anhydride content.

In the present investigation the purified gum acid (uronic acid anhydride content = 23.2%; specific rotation = +10°) was fractionated, by precipitation of the salt of leiocarpan A with cetavlon (cetyl trimethyl ammonium bromide) leaving the salt of leiocarpan B in solution. Essentially the same conditions as reported by McNab[10] were used, and by a stepwise addition of cetavlon accompanied by removal of the insoluble polysaccharide salts which were formed, it was possible to obtain fractions containing leiocarpan A and leiocarpan B as single constituents. A number of intermediate fractions probably containing both polysaccharide components were also obtained and were retained for subsequent refractionation.

After regeneration of the acidic polysaccharides, each was assessed for homogeneity by chromatography on anion exchange cellulose and by this criterion both leiocarpan A and leiocarpan B were shown to be homogeneous. The above method was therefore used for the fractionation of *A. leiocarpus* gum on a preparative scale. Leiocarpan A, the major component, had a

uronic acid anhydride content of 29.4% and specific rotation of $+13.7^{o}$. The minor component, leiocarpan B, had a uronic acid anhydride content of 12.2% and a specific rotation of -5.3^{o}.

Partial Hydrolysis Studies on Leiocarpan A

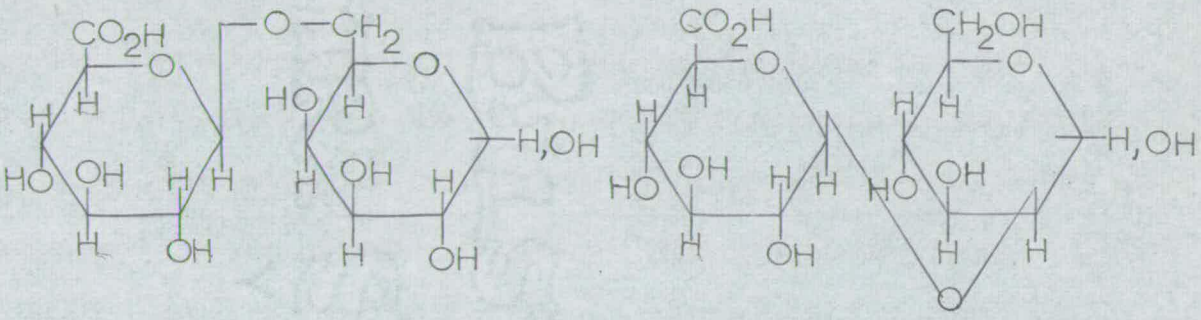
Results of the autohydrolysis of leiocarpan A

Previous investigations on leiocarpan A[10] showed that during the autohydrolysis of an aqueous solution of the gum acid, it was possible to remove the arabinofuranose residues preferentially, leaving a degraded polysaccharide which still contained an appreciable proportion of xylose residues. In the present investigation the course of the autohydrolysis was again followed by measurement of the specific rotation and the reaction was stopped when a value of +27.5° had been reached. The degraded polymer (degraded gum A) was isolated by precipitation in ethanol and was found to have a uronic acid anhydride content of 39.6% and a specific rotation of -8°. (The degraded gum isolated after the previous autohydrolysis, similarly had values of 41.5% and -4.8°.)

A preliminary chromatographic examination of the ethanol soluble cleavage products indicated a complex mixture of both neutral and acidic oligosaccharides in addition to a very large amount of monosaccharides, in particular arabinose. Fractionation of these cleavage fragments by anion exchange chromatography gave rise to a neutral and an acidic fraction.

Two of the sugars in the acidic fraction were respectively chromatographically indistinguishable from samples of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose (i), and 2-O-(β-D-glucopyranosyluronic acid)-D-mannose (ii), and they were characterised as these two aldobiouronic acids after further

examination involving hydrolysis, methylation and measurement of specific rotation. The structures of these two oligosaccharides are therefore

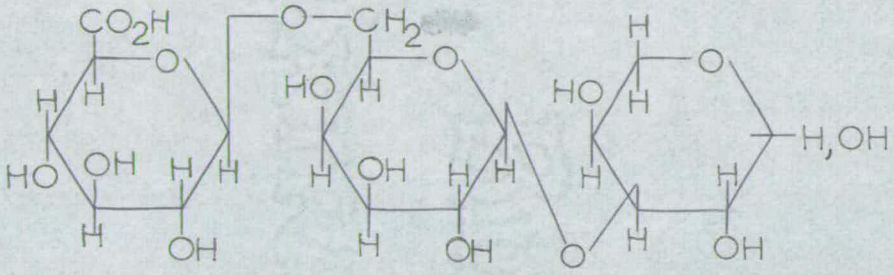


(i) (ii)

The third acidic sugar had the chromatographic mobility of a trisaccharide. Hydrolysis, and methyl ester methyl glycosides formation, followed by hydrolysis, and reduction, indicated that the sugar contained approximately equal proportions of glucuronic acid, galactose and arabinose. In addition, the aldobiouronic acid (i) was recognized as a product of partial hydrolysis. The sugar was methylated by the Kuhn procedure and the product methanolysed. The cleavage products were examined by gas-liquid chromatography and methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid, 2,3,4-tri-O-methyl-D-galactose, and 2,5- and 2,4-di-O-methyl-L-arabinose were detected.

The methylation results therefore show that the trisaccharide is linear. The glucosiduronic acid linkage apparently has the β-configuration due to the detection of (i) as a product of partial hydrolysis. Furthermore in order to accommodate the value of the specific rotation ($+14^{o}$) obtained, the galactosidic linkage has also been assigned the

β-configuration. The structure of this trisaccharide is therefore



(iii)

The first two acidic oligosaccharides (i) and (ii) are therefore identical to the two aldobiouronic acids which were isolated from the partial hydrolysis of unfractionated A. leiocarpus gum[63] and in addition they are, with the trisaccharide, identical to three of the acidic oligosaccharides which were obtained from the graded hydrolysis of leiocarpan A[10].

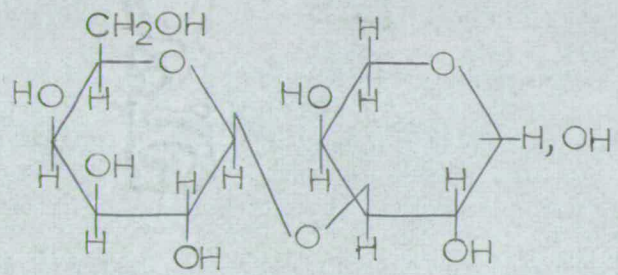
The neutral fraction from the anion exchange column was initially fractionated by adsorption chromatography on charcoal. This gave a preliminary separation into fractions containing monosaccharides, and other fractions which were composed predominantly of oligosaccharides.

Arabinose and xylose were recognized as the most abundant monosaccharides, and were accompanied by a small amount of galactose and trace quantities of rhamnose, ribose and fucose. Further fractionation of these monosaccharides was not attempted.

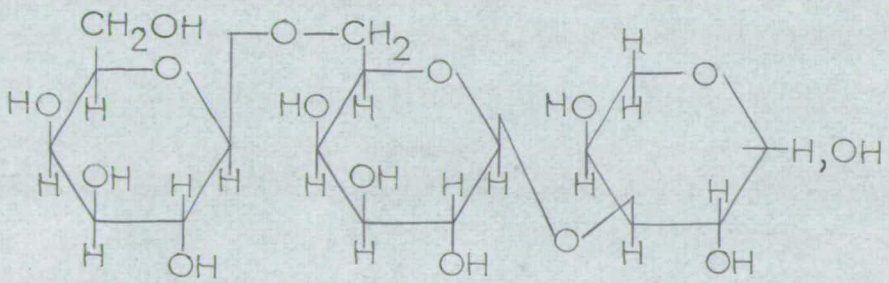
The fractions containing the oligosaccharide fragments were subfractionated by partition chromatography on filter

sheets, and by this method a total of ten chromatographically pure oligosaccharides were obtained, of which two were crystalline sugars and a further five were characterised as known oligosaccharides.

The two oligosaccharides, (iv) and (v), which were obtained as crystalline sugars were identified by comparison with authentic specimens of 3-O-β-D-galactopyranosyl-L-arabinose and the homologous trisaccharide from the series O-β-D-galactopyranosyl-[(1→6)-O-β-D-galactopyranosyl]$_n$-(1→3)-L-arabinose, (n = 0-3), which were previously isolated as crystalline sugars from the unfractionated gum[63]. The structures of these two crystalline sugars are thus



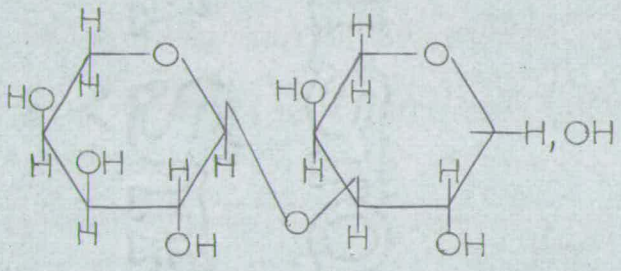
(iv)



(v)

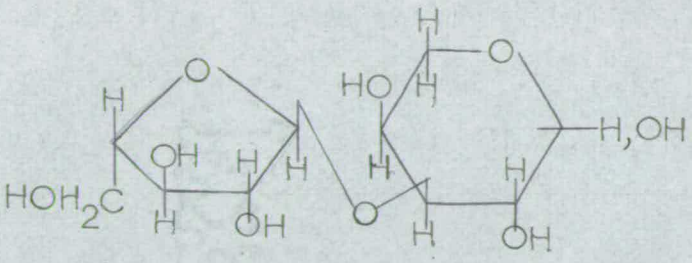
Two of the oligosaccharides, which gave only arabinose on hydrolysis, were characterised. One of these (vi) was chromatographically indistinguishable from 3-O-β-L-arabinopyranosyl-L-arabinose. A sample was methylated by the Haworth procedure and the product was methanolysed. The cleavage

products were examined by gas-liquid chromatography and methyl glycosides of 2,3,4-tri-, 2,4- and 2,5-di-O-methyl-L-arabinose were detected. The β-configuration was assigned to the linkage of this disaccharide on account of its high positive specific rotation (+180°). On the basis of the above evidence this component has the following structure,



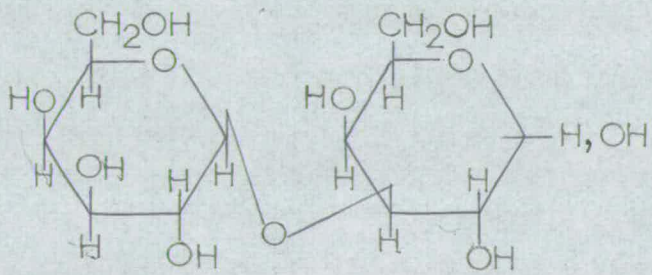
(vi)

The second arabinose bearing oligosaccharide (vii) was chromatographically indistinguishable from 3-O-β-L-arabinofuranosyl-L-arabinose. The sugar was methylated by the Kuhn procedure and the product was methanolysed. The cleavage products were examined by gas-liquid chromatography and methyl glycosides of 2,3,5-tri-, 2,5- and 2,4-di-O-methyl-L-arabinose were detected. The specific rotation (+100°) of the oligosaccharide being similar to that reported for 3-O-β-L-arabinofuranosyl-L-arabinose from unfractionated A. leiocarpus gum[63] but slightly higher than that reported for the same oligosaccharide from A. pycnantha gum[35] and sugar beet araban[89], suggests that the glycosidic linkage has the β-configuration. The structure of this oligosaccharide is therefore



(vii)

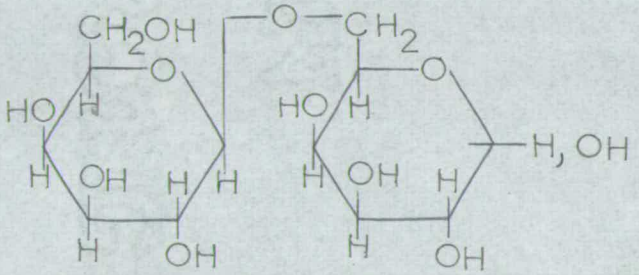
Three oligosaccharides which gave only galactose on hydrolysis were isolated. One of these (viii) was chromatographically indistinguishable from 3-O-galactosylgalactose. The sugar was methylated by the Haworth procedure and the product was methanolysed. Gas-liquid chromatography of the cleavage products indicated the presence of 2,3,4,6-tetra- and 2,4,6-tri-O-methyl-D-galactose. The β-configuration was assigned to the linkage of this disaccharide since 3-O-β-D-galactopyranosyl-D-galactose was isolated as a crystalline sugar from unfractionated A. leiocarpus gum[63]. On the basis of the above evidence the structure of this oligosaccharide is



(viii)

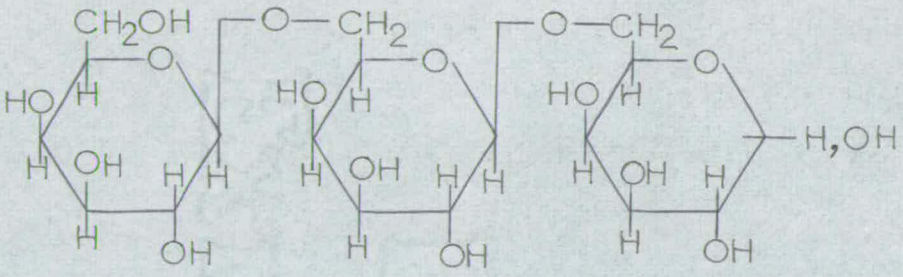
The two other oligosaccharides which gave only galactose on hydrolysis were chromatographically indistinguishable from 6-O-galactosylgalactose (ix) and the homologous trisaccharide (x). The suspected disaccharide was methylated and the product was methanolysed. The cleavage products were examined

by gas-liquid chromatography and methyl glycosides of 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-D-galactose were detected in approximately equal proportions. The β-configuration was assigned to the linkage on the basis of its specific rotation (+26°) which is similar to that of the 1⟶6 galactobiose which was isolated from the unfractionated gum[63] and significantly different from that reported for 6-O-α-D-galactopyranosyl-D-galactose, the major product of the acid catalysed reversion of D-galactose[90]. On the basis of the above evidence the structure of this disaccharide is



(ix)

The suspected homologous trisaccharide (x), isolated in trace amounts, was chromatographically identical to an authentic sample of 1⟶6 galactotriose. Partial hydrolysis gave oligosaccharide (ix) in addition to galactose. While lack of material prevented further characterisation, this component, (x), with the disaccharide, (ix), thus represent the first two members of the series O-β-D-galactopyranosyl-$\left[(1\rightarrow6)\text{-O-}\beta\text{-D-galactopyranosyl}\right]_n$-(1⟶6)-D-galactose, (n = 0-3), which were previously isolated from unfractionated A. leiocarpus gum[63]. The structure of this oligosaccharide is therefore



(x)

Three further oligosaccharides were all isolated in very low yield. One of these on hydrolysis gave rhamnose, xylose and mannose and of the glycitol gave xylose, rhamnose and mannitol, in each case the three components being in approximately equal proportions.

The other two oligosaccharides gave only arabinose on hydrolysis and both were chromatographically distinguishable from arabinose itself. It is interesting to note that one of these had a chromatographic mobility which was identical to that of a suspected arabinobiose which had previously been obtained from the unfractionated gum.

Due to lack of material further investigations on these three components was not attempted and so no structures may be advanced for them.

The above investigations involving hydrolysis, methylation and measurement of specific rotation do not give any information on the ring size of the reducing sugar, but in the case of the two arabinobioses which were characterised as 3-O-β-L-arabinopyranosyl-L-arabinose and 3-O-β-L-arabinofuranosyl-L-arabinose, each is believed to have arisen by the hydrolysis of a furanosidic bond. Evidence supporting this arises from methylation

analysis of the whole gum where it was shown that 1→3 linked arabinofuranose residues, notably shown by the isolation of 2,5-di-O-methyl-L-arabinose, were present in interior parts of the molecule. With respect to the galactosylarabinose and the galactobiosylarabinose, however, it has been shown that the arabinose residue is in the pyranose form. Evidence for this conclusion comes from Smith degradation studies, to be discussed later, where a degraded gum was obtained which gave 3-O-β-D-galactopyranosyl-L-arabinose on hydrolysis, and gave 2,4-di-O-methyl-L-arabinose as the only derivative of arabinose, after methylation.

The acidic tetrasaccharide O-(β-D-glucopyranosyluronic acid)-(1→2)-O-α-D-mannopyranosyl-(1→4)-O-(β-D-glucopyranosyluronic acid)-(1→2)-D-mannose

a) From leiocarpan A

An acidic oligosaccharide, which was obtained from the partial hydrolysis of leiocarpan A[10], was assumed to have the above structure on the basis of the following results.

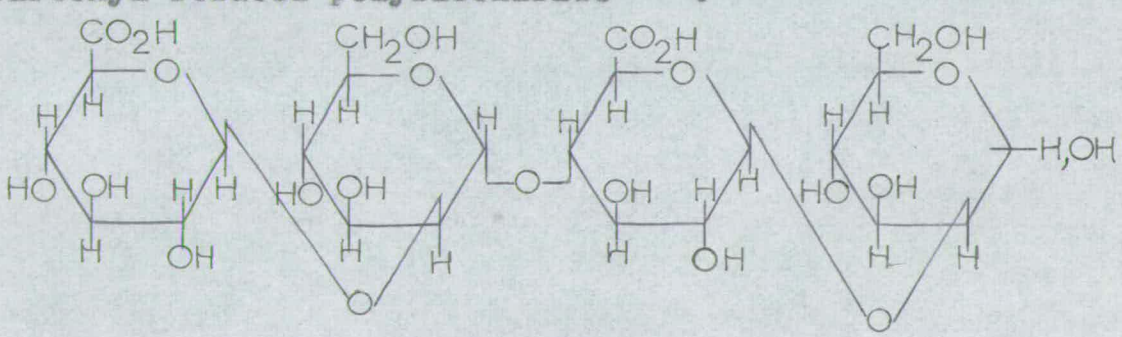
Reduction of the methyl ester methyl glycosides followed by hydrolysis gave glucose and mannose. Hydrolysis gave glucuronic acid, mannose and 2-O-(β-D-glucopyranosyluronic acid)-D-mannose, whilst hydrolysis of the derived glycitol gave the same aldobiouronic acid and its glycitol in addition to glucuronic acid, mannose and mannitol.

The chain length of the oligosaccharide calculated by a

phenol-sulphuric acid determination on the sugar and its glycitol gave a value of 3.9, thus indicating a tetrasaccharide.

Since the above preliminary investigation, the oligosaccharide has been methylated. The fully methylated derivative was reduced and hydrolysed. Fractionation of the hydrolysate was accomplished by partition chromatography on filter sheets and 2,3,4-tri- and 2,3-di-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-mannose were isolated as chromatographically pure sugars. The glucose derivatives were characterised by conversion into their aniline derivatives, which were crystalline. The 3,4,6-tri-O-methyl-D-mannose was obtained as the crystalline sugar.

The structure shown below may be proposed for the acidic tetrasaccharide, the configurations of the glycosidic linkages being based on the earlier characterisation of 2-O-(β-D-glucopyranosyluronic acid)-D-galactose as a partial hydrolysis product from leiocarpan A, and of 2-O-β-D-glucopyranosyl-D-mannose and 4-O-α-D-mannopyranosyl-D-glucose from acetolysis of carboxyl-reduced polysaccharide[10].



b) From gum ghatti

Graded hydrolysis of the gum acid was carried out under

conditions designed to give the maximum yield of the suspected tetrasaccharide. Fractionation of the hydrolysate was accomplished on a column of anion exchange Sephadex and gave a fraction containing neutral sugars and a number of fractions containing acidic sugars. Further fractionation of the acidic sugars, where necessary, by partition chromatography on filter sheets gave five chromatographically pure components. One of these was chromatographically indistinguishable from the acidic oligosaccharide which in the previous investigation on gum ghatti[61] was characterised as O-(β-D-glucopyranosyluronic acid)-(1→2)-O-β-D-mannopyranosyl-(1→2)-D-mannose, and furthermore had the same mobility as the acidic tetrasaccharide from leiocarpan A and was characterised as such after a similar series of experiments to those which were carried out on the acidic tetrasaccharide from leiocarpan A. Due to a lack of sufficient material, however, the hydrolysis products from the reduced methylated oligosaccharide were characterised by paper chromatography of the sugars and by gas-liquid chromatography of the corresponding methyl glycosides, and not as crystalline sugars or their derivatives.

The isolation and characterisation of this component thus disproves the original interpretation[61] which showed it to be a trisaccharide, and shows that at least some, if not all, the D-glucuronic acid and D-mannose residues in gum ghatti, are present in a strictly alternating sequence.

Methylation analysis of leiocarpan A

Methylation of leiocarpan A

Leiocarpan A was methylated with methyl sulphate and sodium hydroxide. Treatment of the partially methylated polysaccharide with silver carbonate gave the silver salt which was methylated to completion by several additions of silver oxide and methyl iodide. The fully methylated polysaccharide (OMe 40.1%; $[\alpha]_D = +1^\circ$) was reduced with lithium aluminium hydride in tetrahydrofuran to give the methylated polysaccharide (OMe 37.4%; $[\alpha]_D = 0^\circ$).

The reduced methylated leiocarpan A was hydrolysed with hydrochloric acid and the hydrolysate was neutralised with silver carbonate. A preliminary examination of the hydrolysate indicated the presence of a large number of methylated sugars. The initial separation of these was accomplished by partition chromatography on a cellulose column. Further fractionation was carried out by adsorption chromatography on charcoal, and/or partition chromatography on filter sheets. In this way twenty-two different methylated sugars were isolated and of these thirteen were characterised by crystallinity of the sugars or of their derivatives, and are as follows,

2,3,5-tri-O-methyl-L-arabinose,

2,3-di-O-methyl-L-arabinose,

2,5-di-O-methyl-L-arabinose,

2,3,4,6-tetra-O-methyl-D-galactose,

2,4,6-tri-O-methyl-D-galactose,

2,4-di-O-methyl-D-galactose,

2-mono-O-methyl-D-galactose,

2,3-di-O-methyl-D-glucose,

3-mono-O-methyl-D-glucose,

3,4,6-tri-O-methyl-D-mannose,

3,4-di-O-methyl-D-mannose,

4-mono-O-methyl-D-mannose,

2,3,4-tri-O-methyl-D-xylose.

The arabinose methyl ethers were all characterised by conversion into the corresponding aldonamides. The 2,4-di- and 2-mono-O-methyl-D-galactose, the 2,3-di- and 3-mono-O-methyl-D-glucose and all three mannose derivatives were obtained as crystalline sugars, the 3,4-di- and 4-mono-O-methyl-D-mannose being further characterised by conversion into their aldonolactones. The 2,3,4,6-tetra- and 2,4,6-tri-O-methyl-D-galactose and the 2,3-di-O-methyl-D-glucose were all characterised as their aniline derivatives. The 2,3,4-tri-O-methyl-D-xylose was characterised by conversion of the sugar into its aldonolactone.

In addition, a number of sugars were characterised by paper chromatography of the sugars, by gas-liquid chromatography of the corresponding methyl glycosides, and in some cases by ionophoresis. The sugars which were characterised in this way were, 2,3,4-tri-, 2,4- and 3,5-di- and 2-mono-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-D-glucose, 2,3-, 2,4- and 3,4-di-O-methyl-D-xylose.

With the exception of those methylated sugars which have

arisen from non-reducing end groups it is difficult to assess the structural significance of certain of the others which were isolated in small amount. For example since the amounts of 3-O-methylglucose and the di-O-methylxyloses are very small in comparison to the amounts of 2,3-di-O-methylglucose and 2,3,4-tri-O-methylxylose respectively, it is possible that the occurrence of these minor components may be a result of under-methylation. Indeed from the results of Smith degradation studies on the carboxyl reduced leiocarpan A, no evidence was obtained for the presence of either glucose or xylose which was resistant to attack by the periodate ion, and so it would appear that the isolation of 3-O-methylglucose and at least the 2,4-di-O-methylxylose is not in fact structurally significant. On the other hand, however, it is known from partial hydrolysis and again from Smith degradation studies that both 3-substituted arabinopyranose and 3-substituted arabinofuranose residues are present in leiocarpan A. The small amounts of some of the di-O-methylarabinoses and probably also the 2-O-methylarabinose are thus structurally significant.

By an extension of the above argument to include the galactose and mannose derivatives, the relative amounts of the methylated sugars which are considered to have structural significance, are given in the following table (Table XXV). The relative amounts of the structurally significant sugars from reduced methylated degraded leiocarpan A[10] are included in the table for comparison.

Table XXV

Methylated monosaccharide	Relative amount of sugar	
	Whole gum	Degraded gum
2,3,5-tri-O-methyl-L-arabinose	++++	not detected
2,3,4-tri-O-methyl-L-arabinose	trace	trace
2,5-di-O-methyl-L-arabinose	+	not detected
2,3-di-O-methyl-L-arabinose	+	not detected
2,4-di-O-methyl-L-arabinose	+	+
2-mono-O-methyl-L-arabinose	trace	trace
2,3,4-tri-O-methyl-D-xylose	++++	++
2,3,4-tri-O-methyl-D-glucose	trace	trace
2,3-di-O-methyl-D-glucose	++++	++++
2,3,4,6-tetra-O-methyl-D-mannose	not detected	+
3,4,6-tri-O-methyl-D-mannose	trace	+++
3,4-di-O-methyl-D-mannose	+++	+++
4-mono-O-methyl-D-mannose	+++	+
2,3,4,6-tetra-O-methyl-D-galactose	trace	+
2,4,6-tri-O-methyl-D-galactose	+	not detected
2,3,4-tri-O-methyl-D-galactose	not detected	+
2,4-di-O-methyl-D-galactose	++	+

From the above results it is apparent that most, if not all, of the arabinofuranose residues and almost half of the xylopyranose residues were removed during the autohydrolysis. On the other hand the relative amounts of arabinopyranose residues are not markedly affected. Furthermore the ~~increase~~ decrease

in the relative amount of 3,4-di- and ~~decrease~~ increase in the amount of 3,4,6-tri-O-methyl-D-mannose on comparing the methylated and methylated degraded gums shows that during formation of the degraded gum substituents have been lost from position 6 of mannose. Similarly the increase in the amount of 2,3,4-tri- with corresponding loss of 2,4-di-O-methyl-D-galactose, suggests that during autohydrolysis, substituents have been lost from position 3 of galactose. The significance of these results in the determination of the structure of leiocarpan A, will be discussed later.

Smith degradation of carboxyl reduced leiocarpan A

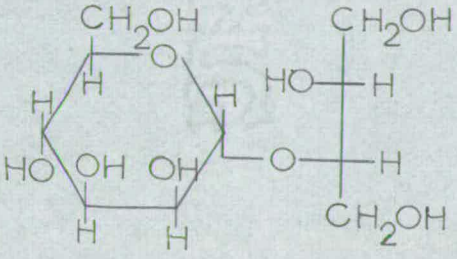
Reduction of leiocarpan A

A sample of leiocarpan A was reduced by the method of Rees and Samuel[91]. The acidic polysaccharide (uronic acid anhydride = 29.4%) was 2-hydroxyethyl esterified on treatment with ethylene oxide. Acetylation of the ester and treatment of the acetate with lithium borohydride in boiling tetrahydrofuran gave reduction of the ester groups with concomitant deacetylation. The carboxyl reduced polysaccharide, which was isolated after dialysis of the acidified reaction mixture and freeze-drying of the non-diffusate, was obtained in good yield (71%). It was found to have a uronic acid anhydride content of 2.4% (by decarboxylation) and no uronic acids were detected by paper chromatography of the hydrolysate.

Smith degradation of the carboxyl reduced leiocarpan A

Carboxyl reduced leiocarpan A was oxidised by an aqueous solution of sodium metaperiodate until the uptake of oxidant had reached a maximum. The polyaldehyde so formed was reduced with sodium borohydride and the resulting polyalcohol was hydrolysed with dilute sulphuric acid, under conditions which were designed to minimize cleavage of glycosidic linkages. Fractionation of the hydrolysate was initially accomplished on Dowex resin[93] and further fractionation by partition chromatography on filter sheets gave chromatographically immobile material and a number of mobile components which consisted mainly of glycerol (from cleaved sugar residues). Also

present was a component which had the chromatographic mobility of a disaccharide. This sugar after analysis by methylation, hydrolysis, measurement of periodate uptake and specific rotation, was characterised as 2-O-α-D-mannopyranosyl-D-erythritol. The α-configuration was assigned to the linkage of this disaccharide on account of the value of the specific rotation (+64°) which was significantly different to that (-35°) which was reported[94] for 2-O-β-D-mannopyranosyl-D-erythritol. The isolation of this oligosaccharide is consistent with the isolation of 4-O-α-D-mannopyranosyl-D-glucose, which was characterised as a product of acetolysis of carboxyl reduced leiocarpan A. The structure of this oligosaccharide is therefore



The chromatographically immobile material, hereinafter referred to as degraded gum B (in contradistinction to degraded gum A which was formed on autohydrolysis of leiocarpan A), was examined by methylation, partial hydrolysis and periodate oxidation procedures.

Partial hydrolysis of degraded gum B, and subsequent examination of the hydrolysate, showed that in addition to monosaccharides, components having identical chromatographic

mobilities to the following oligosaccharides, 3-O-β-D-galactopyranosyl-L-arabinose, O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-L-arabinose, 6-O-β-D-galactopyranosyl-D-galactose and 3-O-β-D-galactopyranosyl-D-galactose were detected. Methylation of degraded gum B by the Kuhn procedure gave a product which was methanolised. The cleavage products were examined by gas-liquid chromatography and methyl glycosides of 2,3,5-tri-, 2,5-, 2,4- and 2,3-di-O-methyl-L-arabinose, 2,4,6-tri-O-methyl-D-mannose, 2,3,4,6-tetra-O-methyl-D-galactose and 1,3,4-tri-O-methyl-D-erythritol were detected. In addition both 2,4-di- and 2-mono-O-methyl-D-galactose were detected by paper chromatography of the hydrolysate of methylated degraded gum B.

Further successive degradations of degraded gum B by the Smith procedure gave, respectively, degraded gums C, D, and E. These degraded gums were examined by partial hydrolysis and methylation techniques. Examination of the partial hydrolysates indicated the presence of the oligosaccharides shown in the following table (Table XXIII).

Table XXIII

Oligosaccharide	Degraded gum			
	B	C	D	E
D-Galp 1→6 D-Gal	+	trace	n.d.	-
D-Galp 1→6 D-Galp 1→3 L-Ara	+	n.d.	-	-
D-Galp 1→3 L-Ara	+	+	+	+
D-Galp 1→3 D-Gal	+	+	+	trace
L-Arap 1→3 D-Man	n.d.	n.d.	trace	+

n.d. = not detected

It should be noted that erythritol was a component of the hydrolysate only of degraded gum B. On the other hand glycerol was produced on hydrolysis of gums C, D and E.

The cleavage products from the respective methylated degraded gums (apart from degraded gum E) were examined by gas-liquid chromatography of the methyl glycosides and by paper chromatography of the sugars. The methylated sugars whose presence was indicated by this method are shown in table XXIV (page 141).

The significance of these Smith degradation results will be discussed later.

Structural features of leiocarpan A

In order to construct the structural features of leiocarpan A from the above experimental data, it is convenient to discuss each part of the molecule in turn.

a. The glucuronomannan chains

From the combined results of partial acid hydrolysis of the gum[10] and acetolysis of the carboxyl reduced gum[64] evidence was obtained whereby it was shown that leiocarpan A had a main chain which was composed of a regularly alternating sequence of D-glucuronic acid and D-mannose. Furthermore since no oligosaccharides have been detected in which mannose is associated with any other sugar, it is assumed that all the mannose residues in leiocarpan A are contained within the main chain. The isolation of smaller amounts of a second aldobiouronic acid, however, shows that glucuronic acid is also associated with galactose. The location and structural significance of this second aldobiouronic will be discussed later.

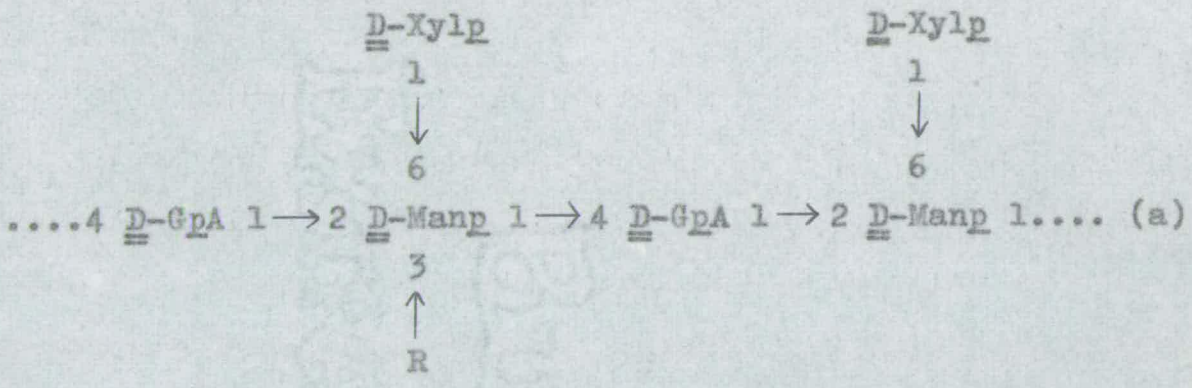
Hydrolysis of the reduced methylated whole gum gave only trace amounts of the 3,4,6-tri-O-methyl-D-mannose. It is apparent therefore that most (probably over 95%), but not all, of the mannose residues carry substituents at position 6, a proportion of these having been lost during the formation of the degraded gum. Furthermore the relatively large amounts of 3,4-di- and 4-mono-O-methyl-D-mannose which were isolated, indicate that approximately two in every five of the mannose residues are doubly branched and carry substituents at C_3 as

well as at C_6. The remainder by the isolation of 3,4-di-O-methyl-D-mannose are shown to carry only one substituent, namely that located at C_6. An examination of the methyl ethers of glucose shows that glucuronic acid in the whole gum occurs mainly (greater than 95%), but not exclusively, as non-substituted chain units. The isolation of trace amounts of 2,3,4-tri-O-methyl-D-glucose shows that a very small proportion is present as non-reducing end groups. The further isolation of a trace quantity of 3-mono-O-methyl-D-glucose would suggest that certain glucuronic acid residues are involved as branching units. Since no periodate resistant glucose residues were detected in the carboxyl reduced gum, however, such units are not considered to have structural significance and so are disregarded.

Hydrolysis of both the reduced methylated and the reduced methylated degraded gums gave 2,3,4-tri-O-methyl-D-xylose as the predominant methyl ether of xylose. Some of the xylose residues were removed during formation of the degraded gum but it is evident that there is no structural difference between those removed and those which remained. It is therefore apparent that the xylose residues exist mainly if not exclusively as non-reducing terminal residues, and furthermore, it is assumed that they are attached directly to the main chain via C_6 of the mannose residue. These conclusions are consistent with the results of Smith degradation studies during which periodate resistant xylose units were not detected.

On the basis of the above results the following partial

structure (a) is a feature of leiocarpan A.



Additional evidence supporting the assumption that almost all mannose residues carry a xylose residue, arises from the observation that there are approximately equal molecular proportions of xylose as its 2,3,4-trimethyl ether and of mannose as the various methyl ethers, in methylated leiocarpan A.

b. The galactan chains

Among others, the following oligosaccharides were isolated as cleavage products from the controlled acid hydrolysis of leiocarpan A.

A. 6-O-β-D-galactopyranosyl-D-galactose.

B. O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-D-galactose.

C. 3-O-β-D-galactopyranosyl-L-arabinose.

D. O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-L-arabinose.

E. 6-O-(β-D-glucopyranosyluronic acid)-D-galactose.

F. O-(β-D-glucopyranosyluronic acid)-(1→6)-O-β-D-galactopyranosyl-(1→3)-L-arabinose.

Oligosaccharides A and B thus represent the first two members of the series.

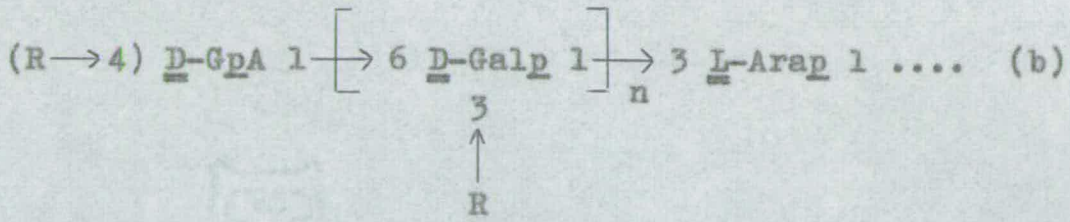
$$\underline{D}\text{-Gal}\underline{p}\ 1\rightarrow\left[6\ \underline{D}\text{-Gal}\underline{p}\ 1\rightarrow\right]_{n}6\ \underline{D}\text{-Gal}\underline{p}\quad(n = 0\text{-}3)\quad\ldots\ldots\quad I$$

and similarly oligosaccharides C and D represent the first two members of the series

$$\underline{D}\text{-Gal}\underline{p}\ 1\rightarrow\left[6\ \underline{D}\text{-Gal}\underline{p}\ 1\rightarrow\right]_{n}3\ \underline{L}\text{-Ara}\quad(n = 0\text{-}3)\quad\ldots\ldots\quad II$$

All the members of the above two series (I and II) were previously isolated from both gum ghatti[60] and from the unfractionated A. leiocarpus gum[63]. It is clear from the isolation of oligosaccharide F that chains of the type II where n = 0, may be terminated by D-glucuronic acid. Oligosaccharide E could then arise from the autohydrolysis of oligosaccharide F, or of higher side chains of the same type. It is probable therefore that most of the 2,3,4-tri-O-methyl-D-glucose which was isolated from the hydrolysate of the reduced methylated degraded gum arose from this unit.

An examination of the methyl ethers of galactose shows that the 2,3,4-trimethyl ether is not detected from either the methylated or methylated degraded gums, and so the galactose residues in the 1→6 linked chains are apparently substituted at position 3. The isolation both of 2,4-di- and 2-mono-O-methyl-D-galactose would tend to support this view. The following (b), although only one of the many partial structures which may be put forward at this point, does agree with the results of partial hydrolysis of the gum, and methylation analysis both of the reduced methylated and reduced methylated degraded gums.



The arabinose in the above partial structure has been assigned the pyranose form, since from Smith degradation studies to be discussed later, it was shown that 3-linked arabinopyranose residues were present in interior parts of the molecule. The length of the 1→6 linked galactan chains is obviously variable. Due to the isolation of the 1→6 galactriose, at least three contiguous galactose residues must be present in some of the side chains. On the other hand the isolation of the aldotriouronic acid, oligosaccharide F, shows that in other cases these side chains may be so short as to have only one galactose residue, and so the suffix n, in partial structure (b) may, on the basis of the above evidence, take the values 1→3. Higher homologues of this 1→6 galactan series have been isolated from the unfractionated A. leiocarpus gum[63] and so it would appear that these higher oligomers are a feature of leiocarpan B. The fact that none were detected here is almost certainly due to the small proportion of galactose (ca. 5%) present in leiocarpan A.

c. The arabinose residues

The majority of these present in leiocarpan A exist as L-arabinofuranose end groups. Hydrolysis of the methylated degraded gum gave evidence for the presence of both 2,3,4-tri- and 2,4-di-O-methyl-L-arabinose. As a result of Smith

degradation studies, to be discussed below, it was shown that the 2,4-dimethyl ether arises from the arabinopyranose residue, which is interposed between the mannose residue in the main chain and the galactan side chain. The isolation of the 2,3,4-trimethyl ether indicates the presence of terminal non-reducing arabinopyranose both in the whole and degraded gums, but as yet no information concerning the location of this moiety in the molecular structure has been obtained.

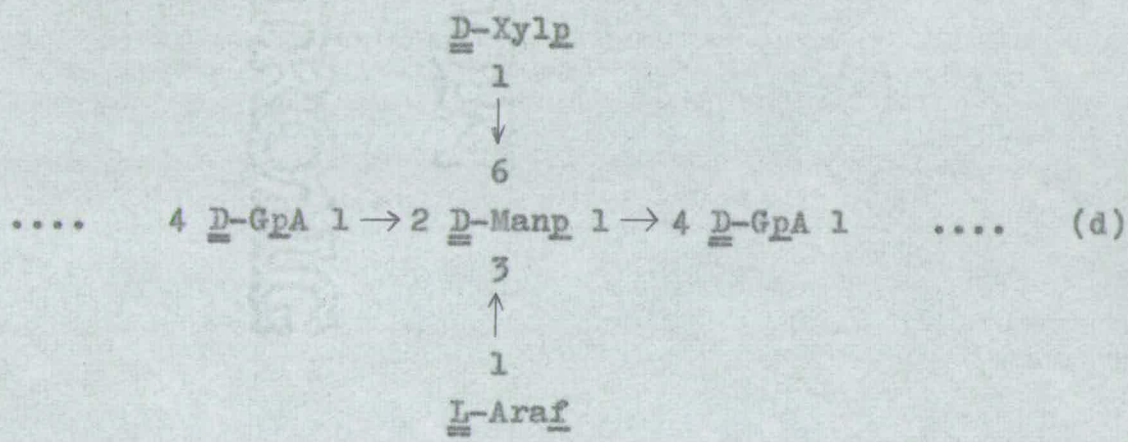
Hydrolysis of the reduced methylated gum gave seven different methyl ethers of arabinose. Of these the 2,3,5-trimethyl ether was predominant, and accounted for over 80% of the mixture. The remainder present mainly as dimethyl ethers indicated the presence of contiguous arabinose residues and this was proved by the isolation of both 3-O-β-L-arabino-pyranosyl-L-arabinose, and 3-O-β-L-arabinofuranosyl-L-arabinose. Furthermore since three degradations of leiocarpan A by the Smith procedure were necessary to remove the last traces of arabinofuranose residues, it is evident that at least three contiguously linked arabinofuranose residues are present at certain parts of the molecule. The following (c), although probably only present in minor amounts, is thus a structural feature,

$$\text{L-Araf } 1 \rightarrow 3 \text{ L-Araf } 1 \rightarrow 3 \text{ L-Araf } 1 \quad .... \quad (c)$$

From the isolation of two further oligosaccharides containing arabinose, it is apparent that contiguous arabinose linked other than 1→3 may also be present. Support for this point stems from the isolation, although in small quantities,

of 2,3- and 3,5-di-O-methyl-L-arabinose from the hydrolysate of reduced methylated leiocarpan A. From the amounts of dimethyl ethers of arabinose isolated, however, the bulk of the arabinofuranose units must be attached to residues other than arabinose.

From methylation analysis of the methylated and methylated degraded gums it is evident that almost 40% of the mannose residues carry substituents at C_3. Since many of these substituents were lost during formation of the degraded gum it was assumed that certain arabinofuranose residues are attached to mannose as in partial structure (d) below:



(d)

For this assumption to be true, the application of one Smith degradation to the carboxyl reduced leiocarpan A should remove the arabinose, glucose (shown as glucuronic acid) and xylose, leaving mannosyl-erythritol. The fact that a substantial quantity of 2-O-α-D-mannosyl-D-erythritol was isolated is positive indication that a partial structure of the above type (d) is a feature of leiocarpan A.

d. The periodate resistant core

The results considered so far indicate that in the gum some galactose and arabinose and approximately half of the

mannose residues are substituted at C_3. Further information concerning the location of these periodate resistant parts of the molecule was sought by the application of the Smith degradation procedure to carboxyl reduced leiocarpan A. Four successive degradations gave respectively degraded gums B, C, D and E. Each of these degraded gums was examined by partial hydrolysis and methylation analysis. The more important cleavage products from these two examinations are given below (Table XXVI).

Table XXVI

Cleavage product	Degraded gum			
	B	C	D	E
a. From methylated derivative				
2,3,5-tri-O-methyl-L-arabinose	+++	++	trace	
2,5-di-O-methyl-L-arabinose	++	trace	n.d.	
2,4-di-O-methyl-L-arabinose	++	++	++	
2,4,6-tri-O-methyl-D-mannose	++	++	++	
2,3,4,6-tetra-O-methyl-D-galactose	+	+	++	
2,4,6-tri-O-methyl-D-galactose	+	+	+	
2,4-di-O-methyl-D-galactose	+	trace	n.d.	
2-mono-O-methyl-D-galactose	++	trace	n.d.	
1,3,4-tri-O-methyl-D-erythritol	+	n.d.	n.d.	
b. From partial hydrolysis				
D-Galp 1→6 D-Gal	+	+	n.d.	-
D-Galp 1→6 D-Galp 1→3 L-Ara	+	n.d.	-	-
D-Galp 1→3 L-Ara	+	+	+	+

Table XXVI (contd.)

Cleavage product	Degraded gum			
	B	C	D	E
D-Galp 1 → 3 D-Gal	+	+	+	trace
L-Arap 1 → 3 D-Man	n.d.	n.d.	trace	+

n.d. = not detected

The reaction sequence may be represented by the following partial structures (i - iii):

Carboxyl reduced leiocarpan A

↓ 1st Smith degradation

Degraded gum B

R → 3 D-Man 1 → 2 erythritol ....(i)

↓ 2nd Smith degradation

Degraded gum C

R′ → 3 D-Man 1 → 2 glycerol ...(ii)

↓ 3rd Smith degradation

Degraded gum D ...(iii)

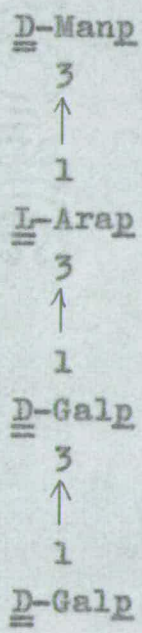
R″ → 3 D-Man 1 → 2 glycerol

↓ 4th Smith degradation

Degraded gum E

Degraded gum D is probably of relatively low molecular weight and is likely to be a mixture of related fragments

rather than a discrete molecular species. The recognition of 2,4-di-O-methyl-L-arabinose, 2,3,4,6-tetra- and 2,4,6-tri-O-methyl-D-galactose, and 2,4,6-tri-O-methyl-D-mannose amongst the cleavage products of the methylated derivative, and of 3-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-galactopyranosyl-L-arabinose and 3-O-L-arabinopyranosyl-D-mannose amongst the partial hydrolysis products of degraded gum D may be accommodated in partial structure (e) if the major part of the degraded polysaccharide is considered as a mixture of fragments, each of which is composed in part of the various possible sequences of sugar residues in this structure.

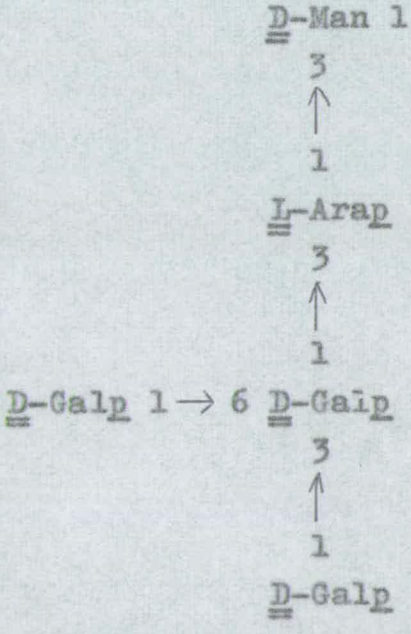


(e)

Partial hydrolysis of degraded gum E to give a relatively diminished amount of 3-O-β-D-galactopyranosyl-D-galactose is consistent with the above partial structure (e), and with the assumption that degraded gum D is in fact a mixture of related fragments. The formulation of partial structure (e) thus

provides the key to the relationship between partial structures (a) and (b) and is based on the fact that the arabinose is in the pyranose form, shown by isolation of the 2,4-dimethyl ether as the only derivative of arabinose from methylated degraded gum D.

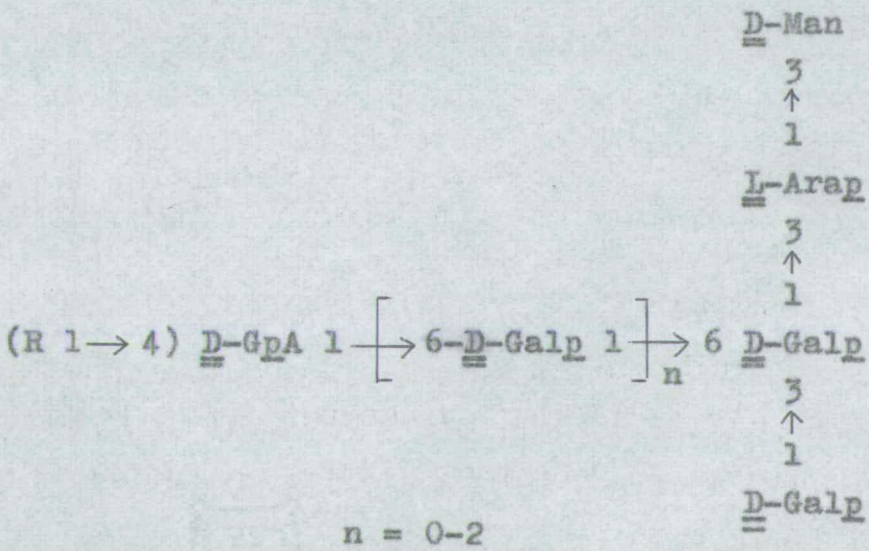
Partial hydrolysis of degraded gum C gave 6-O-β-D-galactopyranosyl-D-galactose in addition to the oligosaccharides isolated from degraded gum D. Examination of the cleavage products from the fully methylated derivative showed that the relative amounts of 2,3,4,6-tetra- and 2,4,6-tri-O-methyl-D-galactose were decreased relative to the amounts of the other sugars which had also been obtained from methylated degraded gum D, and in addition 2,3,5-tri-O-methyl-L-arabinose, 2,4-di- and 2-mono-O-methyl-D-galactose were detected. The observed experimental results from the examination of degraded gum C are represented by the following partial structure (f):



(f)

While it is known that certain of the galactose residues may carry arabinofuranose residues at C_6 and/or C_3, since degraded gum C is a mixture of related fragments and not a discrete molecular species, no attempt has been made to show the attachment of these residues.

Partial hydrolysis of degraded gum B gave O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-L-arabinose in addition to those oligosaccharides indicated from degraded gum C. Examination of the methanolysate of fully methylated degraded gum B indicated the presence of methyl glycosides of a complex mixture of methylated monosaccharides. From the substantial amounts of 2,3,5-tri- and 2,5-di-O-methyl-L-arabinose which were detected it is apparent that the galactan framework is heavily substituted by arabinofuranose residues. This is consistent with an examination of the methyl ethers of galactose, which were detected predominantly as 2,4-di- and 2-mono-O-methyl-D-galactose. By the incorporation of these results with those from the partial hydrolysis, the following partial structure (g) may be advanced for leiocarpan A:



(g)

Although the above partial structure (g) accommodates the results of investigations so far carried out it is only one of the many partial structures which may be put forward. Again as in partial structure (f) no attempt has been made to locate the labile residues. If the above structure is in fact true it is strange that no oligosaccharides of the type D-Galp 1⟶3 D-Galp 1⟶3 L-Ara have been detected, but the apparent absence of such a component may be due to a difference in the rates of hydrolysis of 1⟶3 and 1⟶6 linkages as was found in the case of the mannan from baker's yeast[95,96,97].

In summary, therefore, leiocarpan A has a main chain composed of a regularly alternating sequence of D-glucuronic acid and D-mannose residues. Most, but not all, of the mannose residues carry single xylose units attached at C_6, and almost half are further substituted at C_3. The substituents attached to C_3 of mannose fall into two categories, (a) single arabinofuranose residues, (b) side chains of the galactan type as shown above (partial structure g). The ratio of these two types of side chains is not known.

The galactan type side chains are heavily substituted by labile residues such as arabinofuranose units. While it is known that substituents containing contiguous arabinose residues are present, the exact location of these labile residues is not yet known.

Leiocarpan B

In the fractionation of A. leiocarpus gum by the cetavlon method, it is difficult to obtain substantial amounts of leiocarpan B. On account of this, the structural analyses which have been carried out on this polysaccharide have mainly been on a chromatographic basis.

On total hydrolysis, the gum gave the same monosaccharides as were obtained from leiocarpan A. The relative amounts of these monosaccharides in the two gums are quite different, as shown in the following table.

Fraction	D-Gal	L-Ara	D-Xyl	D-Man	D-G.A	L-Rha
leiocarpan A	+	+++	+++	+++	++++	trace
leiocarpan B	++++	++++	++	++	++	+

Graded hydrolysis of leiocarpan B with N-sulphuric acid at 100° for four and a half hours, gave a complex mixture of neutral and acidic sugars which were fractionated on anion exchange Sephadex. The acidic fraction on examination by paper chromatography against authentic standard sugars indicated the presence of the following acidic oligosaccharides,

D-GpA 1 → 2 D-Man

D-GpA 1 → 6 D-Gal

D-GpA 1 → 2 D-Man 1 → 4 D-GpA 1 → 2 D-Gal

The relative amounts of the two aldobiouronic acids were approximately equal (by visual examination of the paper

chromatogram), and this is different from leiocarpan A, where by a similar examination the relative amount of the mannose containing aldobiouronic acid was very much greater than the other. From the detection of the acidic tetrasaccharide it would appear that in leiocarpan B as in leiocarpan A and in gum ghatti, some, if not all, of the D-mannose and D-glucuronic acid residues are present in a strictly alternating sequence.

An aqueous solution of leiocarpan B was heated on a boiling-water bath. The course of the autohydrolysis, which was continued for an extended period of time, was followed by periodically withdrawing aliquot samples and examining the ethanol soluble cleavage products by paper chromatography against authentic standard sugars. The experiment was carried out in parallel with two others in which leiocarpan B, in one, was replaced by leiocarpan A, and in the other by unfractionated A. leiocarpus gum acid. In each of these experiments the same breakdown pattern was observed, in that the same oligosaccharides were released after similar periods of time. Five neutral oligosaccharides, whose structures were established after the large-scale autohydrolysis of leiocarpan A, were detected and the presence of several others was recognized. The oligosaccharides which were detected were,

3-O-β-L-arabinopyranosyl-L-arabinose

3-O-β-L-arabinofuranosyl-L-arabinose

3-O-β-D-galactopyranosyl-L-arabinose

3-O-β-D-galactopyranosyl-D-galactose

6-O-β-D-galactopyranosyl-D-galactose

A sample of leiocarpan B was methylated on treatment with methyl sulphate and aqueous sodium hydroxide. The partially methylated product was reacted with silver carbonate and the silver salt methylated to completion by four additions of methyl iodide and silver oxide. The fully methylated polysaccharide (OMe 42.1%) was reduced with lithium aluminium hydride in tetrahydrofuran to give the reduced methylated leiocarpan B (OMe 40.5%).

Methylated leiocarpan B and the reduced methylated polysaccharide were methanolysed and the cleavage products were examined by gas-liquid chromatography. The gas chromatograms which were obtained were qualitatively identical to those from a similar investigation of methylated and reduced methylated leiocarpan A. The present evidence indicates therefore that hydrolysis of leiocarpan B gives the same methylated sugars as were obtained from leiocarpan A. Furthermore all the major components present in the hydrolysate of leiocarpan B were previously isolated as cleavage products from methylated leiocarpan A and characterised as crystalline sugars or their derivatives.

The structural features of leiocarpan B

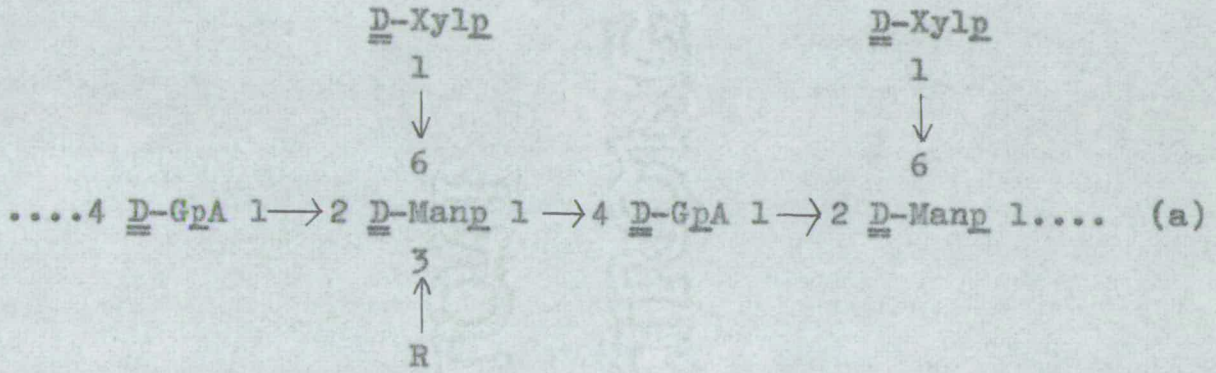
Although investigations on leiocarpan B were mainly based on chromatographic techniques, possible structural features may be proposed in the light of similar results which were obtained for leiocarpan A. The main difference between leiocarpan A and leiocarpan B is the relative proportions of sugars which each contains, particularly with reference to the amounts of galactose, mannose and glucuronic acid. As with leiocarpan A the structural features are best discussed by considering each part of the molecule in turn.

a. The glucuronomannan chains

Leiocarpan B, on hydrolysis, gave a number of acidic oligosaccharides among which a large amount of the aldobiouronic acid, 2-O-(β-D-glucopyranosyluronic acid)-D-mannose was detected. The isolation of this aldobiouronic acid suggests that leiocarpan B like leiocarpan A has main chains composed of mannose and glucuronic acid, and furthermore the presence of the tetrasaccharide O-(β-D-glucopyranosyluronic acid)-(1→2)-O-α-D-mannopyranosyl-(1→4)-O-(β-D-glucopyranosyluronic acid)-(1→2)-D-mannose, in the hydrolysate, indicates that blocks of alternating glucuronic acid and mannose residues are present in the molecule. The presence of 2,3,4-tri- and in much larger amount of 2,3-di-O-methyl-D-glucose in the reduced methylated gum, confirms the assumption that the bulk of the glucuronic acid exists as 4-linked chain units, the remainder being present, but to a lesser extent, as non-reducing terminal

residues. Furthermore the detection of 3,4-di- and 4-mono-O-methyl-D-mannose as the only methyl ethers of mannose shows it occurs both as singly and as doubly branched chain units.

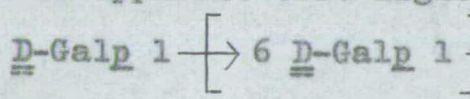
The only xylose methyl ether detected was the 2,3,4-trimethyl derivative. It is thus apparent that xylose exists mainly, if not exclusively, as non-reducing terminal xylopyranose groups, and in comparison with what was found in leiocarpan A, it is assumed that these residues are attached directly to the mannose in the main chain. On the basis of the above evidence the following partial structure (a) may be proposed for leiocarpan B.



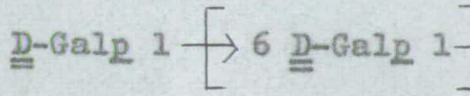
(a)

b. The galactan chains

Whereas galactose is a very minor component of leiocarpan A it is a major constituent of leiocarpan B and although by the detection of 3-O- and 6-O- β-D-galactopyranosyl-D-galactose, evidence has been obtained for only two contiguous galactose residues, it is apparent that higher oligosaccharides from the series

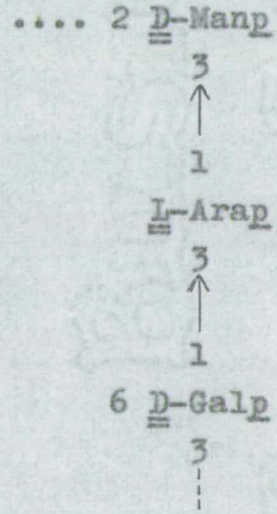


and



are present. This assumption is based on the fact that while all the members of the above two series (I and II) were isolated from the unfractionated A. leiocarpus gum[63] only the lower members were obtained from leiocarpan A. It is therefore evident that the 1→6 linked galactan side chains in leiocarpan B are longer than in leiocarpan A and additional support on this point comes from the fact that no O-(β-D-glucopyranosyluronic acid)-(1→6)-D-galactopyranosyl-(1→3)-L-arabinose was isolated from the partial hydrolysis of leiocarpan B.

The detection of 2,4-di-O-methyl-L-arabinose in the hydrolysate of the methylated gum, and the oligosaccharide 3-O-β-D-galactopyranosyl-L-arabinose from the gum, suggests that, as in leiocarpan A, the mode of attachment of the galactan side chains to the glucuronomannan chain is through an arabinopyranose residue, and thus the following (b) may also be a structural feature of leiocarpan B.



(b)

The two aldobiouronic acids, 2-O-(β-D-glucopyranosyluronic acid)-D-mannose and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose are present in leiocarpan B, but the relative amounts

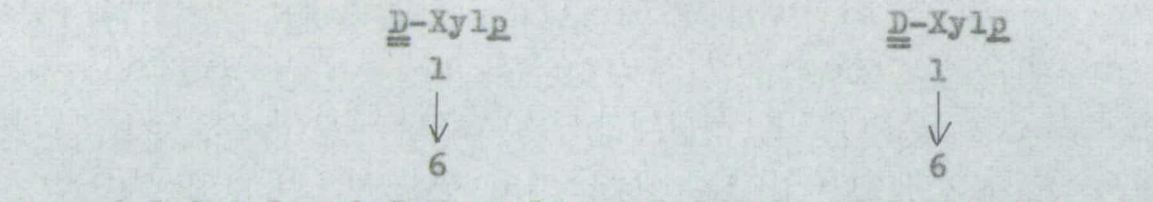
of these are much more equal than was found in leiocarpan A. On the assumption that the galactose bearing aldobiouronic acid terminates the 1→6 linked galactan chains, the additional amount of aldobiouronic acid must be accommodated by the presence of an increased proportion of 1→6 linked chains. The location of these extra chains is not known, but it may be that they replace some of the single arabinofuranose residues which were shown to be attached to the mannose of the main chain in leiocarpan A.

c. The arabinose residues

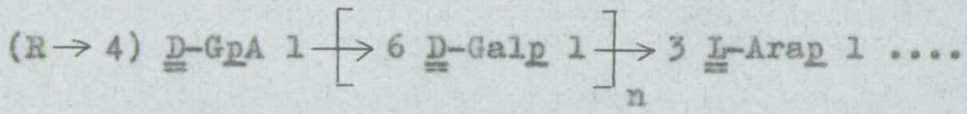
These shown by the detection of large amounts of 2,3,5-tri-O-methyl-L-arabinose, exist predominantly as non-reducing terminal arabinofuranose units. Internal arabinofuranose residues linked 1→3 are also present, this being shown by the detection of the 2,5-dimethyl ether from the hydrolysis of the methylated gum, and of 3-O-β-L-arabinofuranosyl-L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose from partial hydrolysis of the gum acid. Other internal arabinofuranose residues, linked by other means than 1→3, are present, this being shown by the detection of the 2,3- and 3,5-dimethyl ethers. In addition the detection of both the 2,3,4-tri- and 2,4-dimethyl ethers indicates the presence of terminal and internal 1→3 linked arabinopyranose residues.

The main structural features of leiocarpan B may therefore be summarized by the following partial structures:

i)



ii)



$n > 1$

iii) D-Galp 1→3 D-Galp 1 ....

iv) L-Arap 1→3 L-Araf 1 ....

v) L-Araf 1→3 L-Araf 1 ....

The Gums of the Combretaceae

The three gums, gum ghatti, leiocarpan A and leiocarpan B, have all been shown to possess main chains which are composed partly, if not entirely, of a regularly alternating sequence of D-glucuronic acid and D-mannose. In addition all three gums have side chains which are based on galactose. A significant difference between them is, however, the relative propcrtion of galactose which each gum contains. While galactose is only present in a very small amount in leiocarpan A, it is one of the major constituents in the other two gums and this is reflected in the amount, and length, of the $1 \rightarrow 6$ linked oligosaccharides isolated from these gums. On account of this and other features including the uronic acid anhydride content, leiocarpan B bears a greater resemblance to gum ghatti than does leiocarpan A.

The mode of attachment of the galactan side chains has, in gum ghatti and leiocarpan A, been shown to occur through an arabinopyranose residue. While from preliminary evidence it seems possible that this is also the case in leiocarpan B, positive evidence by the isolation of oligosaccharides containing galactose, arabinose and mannose has not yet been obtained.

These three gums therefore represent the first members of the glucuronomannan family of gums. While other gums, notably those from *Virgilia oroboides*[53-57] and certain members of the *Prunus* genus[7], also give rise to 2-O-(β-D-glucopyranosyluronic acid)-D-mannose on hydrolysis, there is as yet little

evidence to suggest the location of this oligosaccharide in these gums. Indeed the failure to detect any 3-O-β-D-galactopyranosyl-L-arabinose from partial hydrolysis of Virgilia oroboides gum, or of 2,4-di-O-methyl-L-arabinose from hydrolysis of the methylated derivative, would suggest that in this gum, at least, significant structural differences exist from those exhibited by the Anogeissus gums.

A fourth member of the Combretaceae, Combretum leonense, has a gum which does not contain mannose but gives the similar aldobiouronic acid 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose on partial hydrolysis. Other features of this polysaccharide, e.g. the 1→6 linked galactose chains and the acid labile periphery, do bear a strong resemblance to the Anogeissus gums, and so if the above aldobiouronic acid arises from the main chain, Combretum leonense gum may have greater structural similarity to the Anogeissus gums than was originally believed.

EXPERIMENTAL

GENERAL METHODS

Paper chromatography

Unless otherwise stated, all qualitative work was carried out by descending chromatography using Whatman No. 1 paper. The chromatograms were developed using the following solvent systems (V/V):

A. ethyl acetate : pyridine : water (10:4:3),
B. ethyl acetate : acetic acid : formic acid : water (18:3:1:4),
C. ethyl acetate : acetic acid : formic acid : water (18:8:3:9),
D. ethyl acetate : pyridine : water (8:2:1),
E. butan-1-ol : ethanol : water (4:1:5, upper layer),
F. methyl ethyl ketone : water : concentrated ammonia (200:17:1),
G. benzene : ethanol : water (169:47:15, upper layer),
H. butan-1-ol : ethanol : water (1:1:1),
I. methyl ethyl ketone : acetic acid : water (9:1:1, saturated with boric acid).

The symbols used in the measurement of relative sugar mobilities and their meanings are as follows,

$$R_F = \frac{\text{Rate of movement of sugar}}{\text{Rate of movement of solvent front}}$$

$$R_{gal} = \frac{\text{Rate of movement of sugar}}{\text{Rate of movement of } \underline{D}\text{-galactose}}$$

$$R_G = \frac{\text{Rate of movement of sugar}}{\text{Rate of movement of 2,3,4,6-tetra-O-methyl-D-glucose}}$$

Unless otherwise stated R_{gal} and R_G values were measured in solvents A and E respectively.

The chromatograms, having been run for a predetermined time, were air dried and the sugars located by spraying the chromatogram with one of the following reagents.

a) Aniline oxalate. Unless otherwise stated, all reducing sugars were detected by spraying with a saturated solution of aniline oxalate in methylated spirits and then heating at 120-130° for about five minutes.

b) p-Anisidine hydrochloride[66]. This was used as a 1% solution in butan-1-ol, as an alternative to aniline oxalate. This spray gives characteristic colours, especially when methylated sugars are present.

c) Alkaline silver nitrate[67]. The presence of polyhydroxy compounds in general may be detected by this reagent which on account of its great sensitivity was used in the detection of trace quantities.

d) Periodate-permanganate spray [68] was used for the detection of both sugars and sugar alcohols.

e) Periodate-Schiff[69]. The presence of sugars which carry an α-glycol group may be detected by this method. Although the sensitivity of the test varies, it is extremely useful especially when formaldehyde is produced after periodate oxidation.

Preparative paper chromatographic separations of sugars were generally carried out on Whatman 3 MM filter sheets, but occasionally other types of paper were used, e.g. Whatman 3 MC and Nos. 17 and 31. All papers were prewashed by boiling methanol in a Soxhlet extractor. The positions of the sugars were determined by cutting off and developing narrow side strips. The appropriate bands of the filter sheet were then cut out and the sugars eluted, generally by a solution of 20% ethanol in water.

Paper electrophoresis[70] was carried out qualitatively on Whatman No. 1 paper in borate buffer (pH 10) at a potential of 350 volts for six hours. The sugars were located by spraying the air dried electrophoretogram with p-anisidine hydrochloride [1% in a solution of glacial acetic acid in butan-1-ol (10%, V/V)]. This method enabled the characterisation of certain sugars by means of their Mg value, which is the ratio of the distance travelled by the sugar to that travelled by glucose.

Evaporations were carried out under reduced pressure at or below 40^{o}.

Optical rotations were measured at about 18^{o} in aqueous solution (unless otherwise stated) using the D-line of sodium as light source.

Cellulose columns were packed as a slurry in acetone. The packed column was washed with acetone containing an increasing amount of water, water and finally with the solvent to be used.

DEAE-Cellulose columns[14]. The ion exchange cellulose was washed alternatively with 0.5 N-hydrochloric acid and 0.5 N-sodium hydroxide. The powder was stirred with the acid (or base), for several minutes, centrifuged, and the supernatant turbid solution decanted off. The cellulose was then washed with water until free of acid (or base) and packed as a slurry in water. The initial generation and subsequent regeneration of the cellulose in the phosphate form was achieved by eluting the column with 0.5 M sodium dihydrogen phosphate (buffered at pH 6 by the addition of sodium hydroxide). Equilibration of the column was by 0.05 M sodium dihydrogen phosphate solution of the same pH. The polysaccharide was taken up in a little water and allowed to soak into the column overnight. The rate of elution was generally adjusted to 40-50 ml./hour.

DEAE-Sephadex columns. Diethylaminoethyl-Sephadex (A 25) was allowed to swell in water and all fine material removed by decantation. The resin was washed with 0.5 M hydrochloric acid and then 0.5 M sodium hydroxide, before generating in the formate form by stirring with 15% formic acid. The resin was packed in a column plugged with glass wool and washed with distilled water until the eluate was free of formic acid.

Charcoal-Celite columns[71] were used in the separation of methylated monosaccharides and in the fractionation of mixtures of oligosaccharides. The Celite was washed with concentrated hydrochloric acid : water (1:1), allowed to stand overnight, filtered and washed with distilled water until free of chloride

ions. The charcoal was washed six times in boiling distilled water, fine materials being decanted off between washings. The mixture of charcoal : Celite (1:1) was packed into columns as a water slurry and thoroughly washed with water prior to using.

Hydrolyses

a) Hydrolysis with sulphuric acid

Small scale hydrolyses were carried out on 1-5 mg. of sample in 0.5 - 2 ml. of N-sulphuric acid in a sealed tube at 100°. The duration of the hydrolysis varied from four hours for a neutral oligosaccharide or sugar glycoside to eight hours for acidic polysaccharides. Neutralisation of sulphuric acid was effected by adjusting to pH 5 by the addition of saturated barium hydroxide and finally to pH 7 on the addition of barium carbonate, this process being used in the case of neutral sugar residues. When acidic sugars were present in the hydrolysate, Amberlite LA-1 resin (5% in light-petroleum or chloroform) was used in place of barium carbonate to effect the final stage of the neutralisation. The basic solution was then treated with Amberlite IR-120(H^+) cationic exchange resin and concentrated to give a syrup which was examined by paper chromatography.

b) Hydrolysis with hydrochloric acid

Samples of methylated polysaccharides were dissolved in 2 N-hydrochloric acid (1% solution) and allowed to stand at room temperature for two days. In order to keep the polysaccharide in solution the temperature was raised very slowly

until having reached 100°, the hydrolysis mixture was diluted by an equal quantity of water. Heating at 100° was then continued until no further change in the optical rotation was observed. The solution was then neutralised with silver carbonate and insoluble salts removed at the centrifuge. By passing hydrogen sulphide through the centrifugate, colloidal silver was converted to silver sulphide which was removed by filtration through glass fibre paper. The filtrate was concentrated to a syrup which was examined chromatographically in solvents E, F and G.

De-ionization. Removal of cations from sugar solutions was effected by treatment with Amberlite IR-120(H^+) cationic exchange resin. Anions were removed on treatment with Duolite A-4 or Amberlite LA-1 (5% in chloroform or light petroleum) liquid resin.

Methanolyses were carried out by heating 1-2 mg. of sample in 0.5 - 1 ml. of dry methanolic hydrogen chloride (4%) in a sealed tube at 100° for periods varying from four hours for neutral methylated disaccharides to eighteen hours for methylated acidic polysaccharides. The solution was cooled, neutralised with silver carbonate and centrifuged to remove silver salts. The centrifugate was carefully concentrated to give the methyl glycosides.

Small scale methylations[72] were carried out by dissolving the sugar (0.5 - 2.0 mg.) in N,N dimethyl formamide (0.2 ml.) and

adding methyl iodide (0.2 - 0.4 ml.) and silver oxide (1-2 mg.). The mixture was shaken in the dark for eighteen to twenty-four hours. The silver salts were removed at the centrifuge and the solution concentrated to small volume. The dimethyl formamide was removed azeotropically with redistilled toluene. The resultant syrup was dried in a vacuum desiccator prior to methanolysis and subsequent examination by gas-liquid partition chromatography.

Gas-liquid partition chromatography[73] was carried out qualitatively on a "Pye Argon Chromatograph" according to the method of Bishop and Cooper[74]. Separations were achieved by employing various stationary liquid phases which were supported on acid washed Celite (80-100 mesh). The liquid phases used were as follows,

a) 15% by weight of butan-1,4-diol succinate polyester,

b) 10% by weight of polyphenyl ether m-bis-(m-phenoxyphenoxy) benzene ,

c) 3% by weight of neopentylglycol adipate polyester,

d) 11% by weight of diethyleneglycol succinate polyester.

Operating temperatures were a) 175°, b) 200°, c) 150°, d) 175°.

Retention times (T) of the methyl glycosides are given relative to the mobility of β-methyl-2,3,4,6-tetra-O-methyl-D-glucopyranoside.

Preparative gas-liquid chromatography used in the separation of trimethyl pentoses and their lactones was carried out

on an "Aerograph autoprep" using a column of 5% XE-60 on Gas-chrom Z, at 175°.

Demethylations were carried out by two alternative methods depending on whether (a) or not (b) the methylated sugar or its methyl glycoside was soluble in dichloromethane.

a) The methylated sugar was dissolved in dichloromethane and treated with boron trichloride[75]. In certain cases the solubility in dichloromethane was enhanced by first forming the methyl glycoside of the sugar.

b)[76] The sugar (2-4 mg.) was heated with hydriodic acid (1 ml.) in a sealed tube for eight minutes at 100°. The contents were immediately diluted with water (10 ml.) and the solution neutralised by the addition of silver carbonate. A small quantity of charcoal was stirred in and all insoluble materials removed at the centrifuge. The solution was treated with hydrogen sulphide which thus converted colloidal silver to silver sulphide which was removed by filtration through glass fibre paper. Concentration of the filtrate gave a syrup which was examined by paper chromatography in solvents A and B.

Reduction techniques

a) Sugar to sugar alcohol[77]. This was achieved by the addition of sodium borohydride to an equal amount of sugar dissolved in water. The solution was allowed to stand overnight after which cations were removed on treatment with Amberlite IR-120(H^+) cationic exchange resin. Concentration gave a syrup which was exhaustively evaporated (6 times) with

methanol to remove borate ions, and finally examined by paper chromatography.

b) Aldobiouronic acid. The aldobiouronic acid was converted to the corresponding methyl ester methyl glycosides by heating in methanolic hydrogen chloride (4%) in a sealed tube at 100° for six hours. After neutralisation with silver carbonate and evaporation of the solvent, the products were dissolved in water and treated with sodium borohydride as in a) above. This treatment reduced the methyl ester and produced a mixture of methyl glycosides which were hydrolysed by N-sulphuric acid to give the neutral monosaccharides.

c) Methylated polysaccharide. Tetrahydrofuran was purified by standing over sodium wire for forty-eight hours, followed by distillation over lithium aluminium hydride. Methylated polysaccharide (100 mg.) was dissolved in tetrahydrofuran (3 ml.) and lithium aluminium hydride (100 mg.) in tetrahydrofuran (3 ml.) was added. After thirty minutes at room temperature the mixture was stirred under reflux for three hours. Excess of hydride was destroyed by the addition of aqueous ethyl acetate. The resulting mixture was adjusted to pH 4 by the addition of 2 N-sulphuric acid and the reduced methylated polysaccharide extracted with chloroform.

Analytical techniques

Methoxyl determinations[78] were carried out by the semi-micro Zeisel method, using a 10% solution of sodium antimony tartrate as scrubber. All methylated samples were soluble in the

hydriodic acid and so other solvents were not required.

Small scale periodate oxidations of methylated sugars were carried out by the method of Lemieux and Bauer[79].

Phenol sulphuric acid reagent[17] was used to determine total sugar content.

Estimation of uronic acid anhydride content

a) Carbazole colorimetric reagent[80] was used on the micro-scale to determine uronic acid content.

b) Decarboxylation[18]. In this method the polysaccharide (ca. 40 mg.) was treated with 19% hydrochloric acid. The amount of carbon dioxide released gave a measure of the uronic acid anhydride content of the polysaccharide.

Determination of the amount of periodate consumed was estimated spectrophotometrically[81].

Formation of derivatives

Anilides of the methylated sugars were prepared by refluxing a solution of the sugar in ethanolic aniline (equimolecular proportions of sugar and aniline) for one to six hours in the dark. Evaporation of the solvent gave the crystalline anilide which, unless otherwise stated, was recrystallised from ethyl acetate.

Lactones of the aldonic acids of methylated sugars were prepared as follows. The methylated sugar (10-100 mg.) was dissolved in bromine water (2-3 ml.) and the solution kept in the dark for three days. Bromine was then removed by aeration

and the solution neutralised by the addition of silver carbonate. Insoluble silver salts were removed by filtration and the filtrate evaporated to dryness. The lactone was extracted with acetone. The acetone extracts were evaporated to give a syrup from which the lactone crystallised and was re-crystallised from the given solvent.

Aldonamides were prepared from the corresponding lactones which had been previously dried in a vacuum desiccator. The lactone (10-100 mg.) was dissolved in dry methanolic ammonia (1-5 ml., 8%) and allowed to stand in the refrigerator for two days. On evaporation of the solvent, the amide crystallised and was re-crystallised from the given solvent.

Purification of solvents was as described below:

a) Tetrahydrofuran and dimethoxyethane were allowed to stand over sodium wire for one day, filtered and distilled over lithium aluminium hydride.

b) Dimethyl sulphoxide was distilled over calcium hydride.

c) Dimethyl sulphate was redistilled and then allowed to stand over potassium carbonate until it was no longer acid to congo red paper.

d) Aniline was distilled in the dark in an atmosphere of carbon dioxide.

e) Methyl iodide was distilled over silver oxide.

f) Light petroleum was shaken with concentrated sulphuric acid (10%, V/V) for twenty-four hours, washed free of acid with distilled water, dried over anhydrous sodium sulphate and

distilled.

g) Butan-1-ol was refluxed with potassium hydroxide for two hours, and then distilled.

h) Pyridine was refluxed for three hours over potassium hydroxide and then distilled.

EXPERIMENTAL

Isolation and purification of Anogeissus leiocarpus gum

The crude gum was obtained in the form of nodules of varying size and colour, which were contaminated by pieces of bark. The gum (25 g.) was crushed and stirred in water (2 l.) for two days after which all the gum had dissolved. Pieces of bark and other extraneous materials were removed by centrifugation followed by filtration of the centrifugate through glass fibre paper. The clear filtrate was acidified by the addition of concentrated hydrochloric acid (40 ml.) and the solution was poured slowly with stirring into ethanol (8 l.). The precipitated polysaccharide was dissolved in water (1 l.) containing concentrated hydrochloric acid (20 ml.) and the solution was slowly poured into ethanol (4 l.). The re-precipitated polysaccharide was dissolved in water (1 l.) and chloride ions removed by dialysis against tap water for five days, concentrated (500 ml.) and freeze-dried to give the complex gum acid (21.4 g.). $[\alpha]_D = +10.2^\circ$ (c = 1.24). Uronic acid anhydride content = 23.2% (by decarboxylation).

Fractionation of complex gum acid

The complex gum acid (21.4 g.) was dissolved in water (2 l.) and a 20% aqueous solution of cetavlon (cetyltrimethylammonium bromide) (50 ml.) was added with stirring. The solution became extremely cloudy and was allowed to stand overnight. The precipitated salt was removed at the centrifuge,

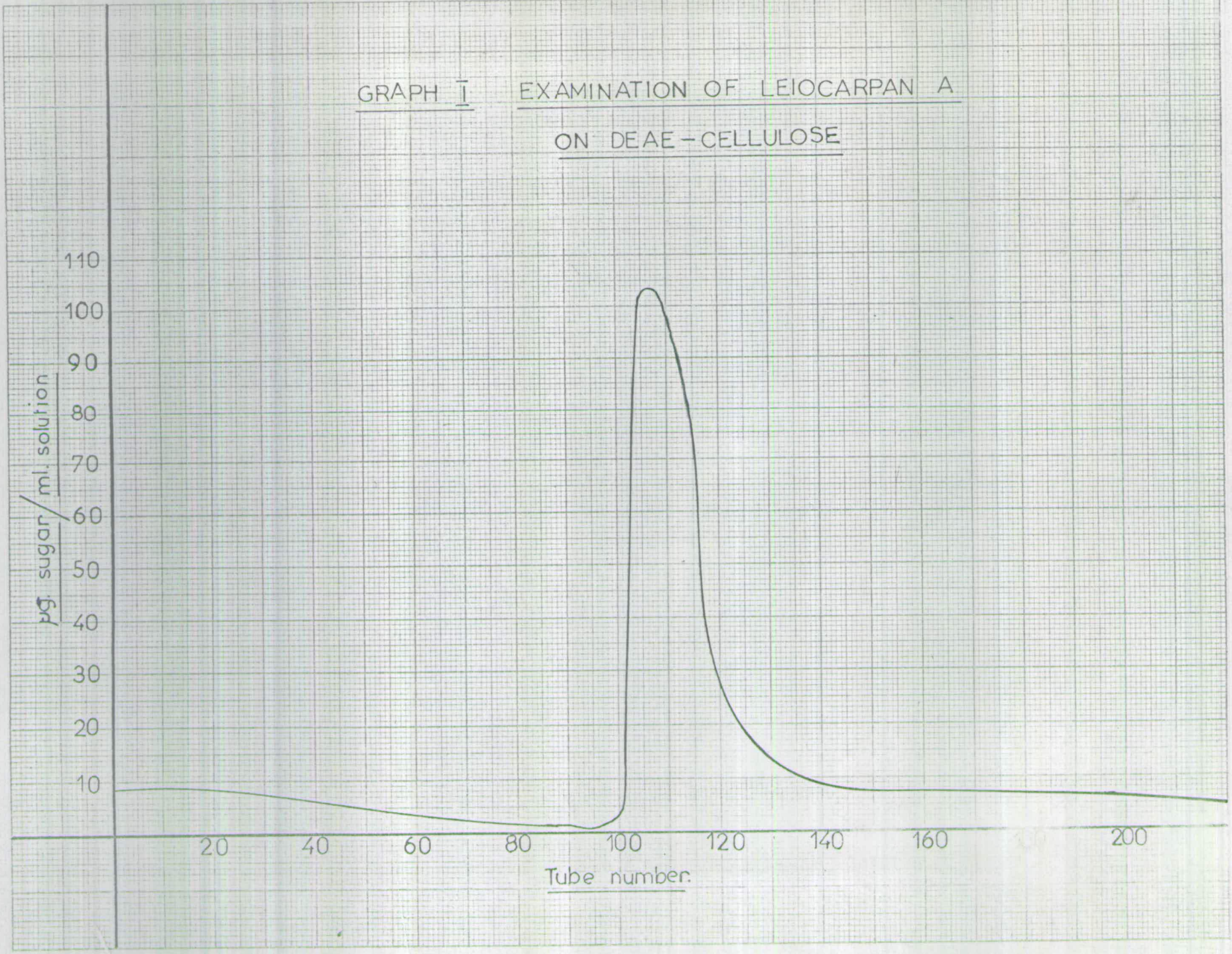
washed with water, and decomposed by the addition of excess aqueous sodium chloride solution (10%). The resulting solution was poured into ethanol (3 volumes) with stirring to give fraction I which was precipitated. This fraction was re-dissolved in water, dialysed, concentrated, and freeze-dried. Yield = 5.4 g.

The supernatant solution obtained after removal of the first salt precipitate was treated with a further addition of cetavlon solution (50 ml.). The precipitated salt was decomposed and the polysaccharide purified as above to give fraction II (4.9 g.).

After three further precipitations to give fractions III, IV and V (3.4 g., 3.6 g. and 0.6 g. respectively), a clear solution was obtained which did not give a precipitate on further addition of cetavlon solution (50 ml.). This clear solution was passed through a column of Amberlite resin IR 120(H^+) to remove cations, dialysed against tap water for five days, concentrated (100 ml.) and freeze-dried to give fraction VI (1.7 g.).

A summary of the above results is given in the following table.

GRAPH I EXAMINATION OF LEIOCARPAN A ON DEAE-CELLULOSE



Fraction	Cetavlon added (ml.)	Uronic acid anhydride content	$[\alpha]_D$	Weight (in g.)
I	50	29.4%	+ 13.7°	5.4
II	50	30.7%	+ 14.1°	4.9
III	50	-	-	3.4
IV	50	-	-	3.6
V	50	-	-	0.6
-	50	-	-	-
VI	residue	12.2%	- 5.3°	1.7

Fractions I and II were combined to give leiocarpan A.

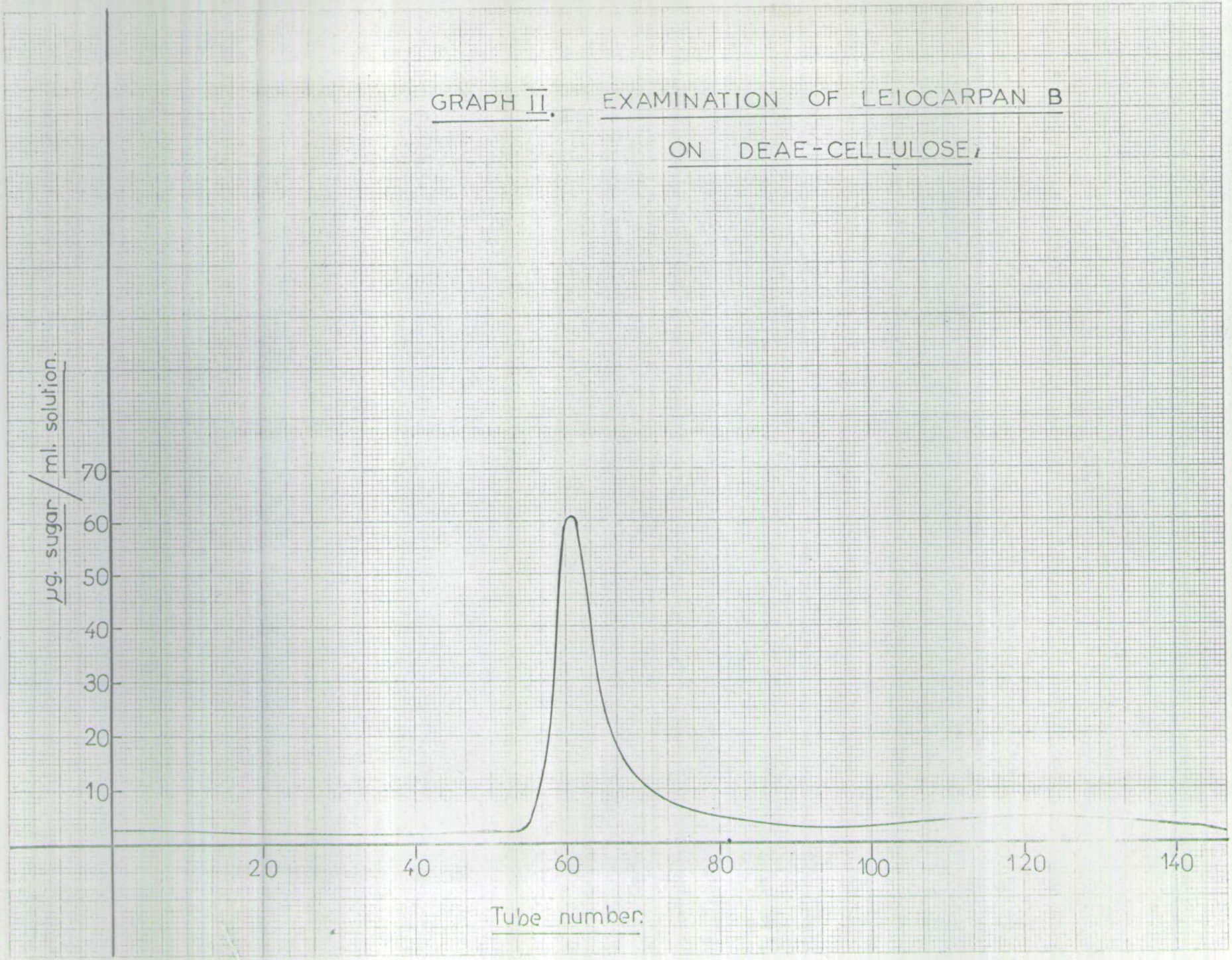
Fractions III, IV and V were combined and kept for subsequent refractionation.

Fraction VI was taken as leiocarpan B.

Assessment of homogeneity of leiocarpan A

Leiocarpan A (0.04 g.) in water (10 ml.) was stirred with Amberlite resin to ensure complete removal of metal ions. After filtration the solution was pipetted on to the top of a DEAE-cellulose column (4.2 x 30 cm.; 30 g.) which had been prepared in the normal way. After allowing the polysaccharide to remain on the column overnight, the column was successively developed with the following eluants:

GRAPH II. EXAMINATION OF LEIOCARPAN B ON DEAE-CELLULOSE.



a) 0.05 M (500 ml.)
b) 0.10 M (500 ml.)
c) 0.25 M (500 ml.)
d) 0.50 M (500 ml.)

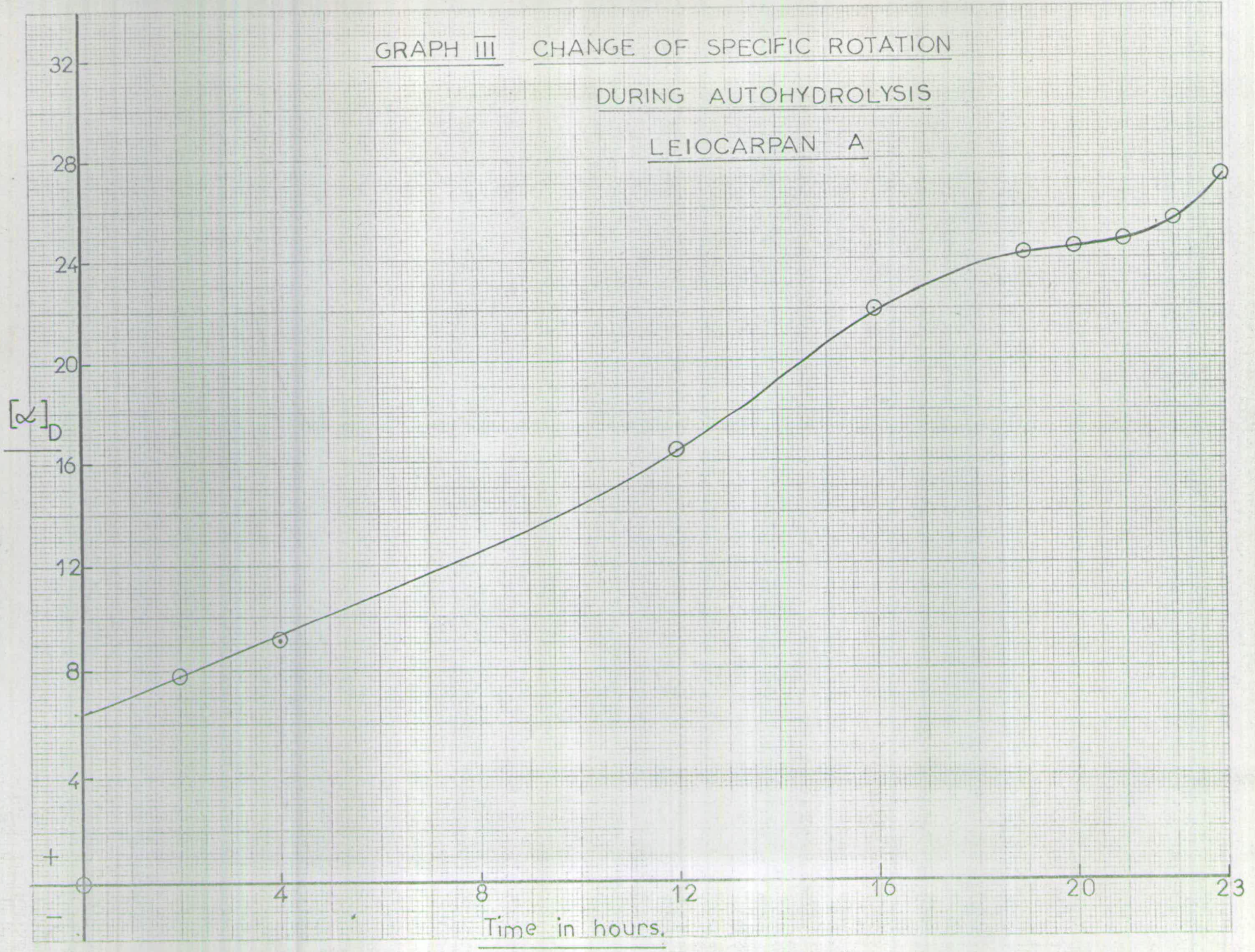
} of sodium dihydrogen phosphate buffered at pH 6

e) 0.50 M (1 l.) potassium chloride solution

The flow rate of the column was adjusted to 50 ml. per hour, fractions being collected every twenty minutes. The amount of polysaccharide in each tube was estimated by the phenol-sulphuric acid method, using a standard curve based on D-galactose. A graph of polysaccharide content against tube number and strength of buffer is shown on Graph I. Only one fraction was obtained and was eluted with the 0.5 M phosphate buffer.

Assessment of homogeneity of leiocarpan B

Leiocarpan B (0.015 g.) was examined on DEAE-cellulose by an exactly analagous method to the above. The result is shown on Graph II. Only one fraction was obtained and was eluted with the 0.25 M phosphate buffer.


GRAPH III CHANGE OF SPECIFIC ROTATION
DURING AUTOHYDROLYSIS
LEIOCARPAN A
32
28
24
20
16
12
8
4
+
−
[α]D
4
8
12
16
20
23
Time in hours.

Autohydrolysis of leiocarpan A

A solution of the purified gum acid (10 g.) in water (500 ml.) was heated on a boiling-water bath until the specific rotation of the solution (see Graph III) had reached +27.5°. (Previous experiments[10] showed that the arabinose release was at a maximum at this point while still leaving an appreciable amount of xylose in the degraded polysaccharide.) The autohydrolysate was then poured into ethanol (2 l.) so precipitating the degraded gum which was removed at the centrifuge, dissolved in water (100 ml.) and re-precipitated in ethanol (500 ml.). After removal at the centrifuge it was dissolved in water (100 ml.) and freeze-dried (4.26 g.). Uronic acid anhydride content = 39.6% (by decarboxylation); $[\alpha]_D = -8^\circ$ (c = 0.4).

The ethanolic centrifugates were combined and concentrated to a syrup (4.7 g.) which on chromatographic examination in solvents A and B was shown to contain a complex mixture of both neutral and acidic oligosaccharides in addition to the monosaccharides arabinose, xylose and galactose.

Separation of neutral and acidic sugars

The syrup (4.7 g.) was placed on a DEAE-sephadex column (30 g.; 3 x 20 cm.) which had been prepared in the formate form. The sugars in water (30 ml.) were applied to the column in three batches, each batch being washed in with water (10 ml.) before the next was added. The sugars were then allowed to soak well into the column by allowing it to stand overnight,

after which the neutral components were eluted with carbonate free water (ca. 1500 ml.) until the eluate gave a negative reaction to the phenol-sulphuric acid reagent. This fraction was then concentrated to give a dried syrup (4.3 g.).

The acidic sugars remaining on the column were eluted by 3% formic acid solution (1 l.), elution again being continued until a blank reaction with the phenol-sulphuric acid reagent was obtained. Further elution at this point using 10% formic acid solution (500 ml.) failed to yield any chromatographically mobile oligosaccharides.

The fraction eluted by the 3% formic acid solution was concentrated to small volume (30 ml.) and the formic acid removed on extraction with ether, in a liquid-liquid Soxhlet, overnight. The formic acid free solution was then concentrated to give a dried syrup (0.038 g.). After preliminary chromatographic examination this fraction was separated into three sugars on thick paper in solvent B.

Oligosaccharide A_1

This sugar [0.020 g.; $[\alpha]_D = +14^\circ$ (c = 1.0)] was chromatographically pure in solvent B (R_{gal} = 0.08) and in solvent C (R_{gal} = 0.40) being indistinguishable from O-(β-D-glucopyranosyluronic acid)-(1⟶6)-O-β-D-galactopyranosyl-(1⟶3)-L-arabinose.

Total hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvents A, B and C, indicated the presence of arabinose, galactose, glucuronic acid and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. Reduction of

the methyl ester methyl glycosides with sodium borohydride, followed by hydrolysis gave galactose, glucose and arabinose in approximately equal proportions (visual inspection of the paper chromatogram).

A small sample (5 mg.) was methylated by the Kuhn procedure and the product was methanolysed. Gas-liquid partition chromatography of the derived methyl glycosides on system (C) indicated the presence of the following methylated sugars, with their relative retention times in parentheses: 2,5- ($\underline{T}$ = 1.52), and 2,4-di-O-methyl-L-arabinose ($\underline{T}$ = 2.04), 2,3,4-tri-O-methyl-D-glucuronic acid ($\underline{T}$ = 2.32 and 3.04), and 2,3,4-tri-O-methyl-D-galactose ($\underline{T}$ = 7.02).

Oligosaccharide A_2

This sugar [0.008 g.; $[\alpha]_D = +6^\circ$ ($\underline{c}$ = 0.8)] was chromatographically pure and indistinguishable from 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, in solvent B (R_{gal} = 0.18) and in solvent C (R_{gal} = 0.50).

A sample of the sugar (2 mg.) was subjected to total hydrolysis and examination of the hydrolysate in solvents A, B and C indicated the presence of glucuronic acid, glucurone and galactose.

The remainder of this sugar was methylated via the Kuhn procedure. The product was methanolysed and examination of the methyl glycosides on system (C) indicated the presence of the following sugars, 2,3,4-tri-O-methyl-D-glucuronic acid ($\underline{T}$ = 3.14 and 2.39) and 2,3,4- ($\underline{T}$ = 6.96) and 2,3,5-tri-O-methyl-D-galactose ($\underline{T}$ = 4.07).

Oligosaccharide A_3

The sugar [0.005 g.; $[\alpha]_D = -8.9^\circ$ (c = 0.3)] was chromatographically pure in solvent B (R_{gal} = 0.39) and in solvent C (R_{gal} = 0.67) being indistinguishable from 2-O-(β-D-glucopyranosyluronic acid)-D-mannose in both solvents. A small sample (1 mg.) was hydrolysed and the hydrolysate on examination in solvents A and B showed the presence of glucuronic acid, glucurone and mannose.

A further sample of the sugar (2 mg.) was methylated by the Kuhn procedure and the product was methanolysed. Gas-liquid partition chromatography of the methyl glycosides on system (C) indicated the presence of the following sugars: 2,3,4-tri-O-methyl-D-glucuronic acid (T = 3.12 and 2.39) and 3,4,6-tri-O-methyl-D-mannose (T = 2.78).

Examination of the neutral fraction

From a preliminary chromatographic examination of this neutral fraction evidence was furnished for the presence of arabinose, but due to the very large amount of this component, little information concerning any other component was obtained.

The syrup was placed on a charcoal-Celite column (200 g. powder; 6 x 35 cm.) and the column was eluted first with water (6 l.), and later with water containing a gradually increasing concentration of ethanol (0% → 10%). The water washings were collected in batches (4 x 1500 ml.). The neutral oligosaccharides were then eluted by the solution of ethanol in water, fractions (20 ml.) being collected every thirty minutes.

The elution pattern was followed by the chromatographic examination of every third tube in solvents A and B. The following fractions were obtained (Table XII).

After fraction 11 the column was eluted with a 20% solution of ethanol in water (2 l.) but this failed to yield any further oligosaccharides.

Table XII

Fraction	Eluant	Weight in g.	Contents
1a	Water	1.460	monosaccharides
1b	"	2.039	monosaccharides
1c	"	0.120	monosaccharides
1d	"	0.100	monosaccharides
2	0→1.5% ethanol	0.030	arabinose + oligosaccharide A
3		0.025	oligosaccharide A
4		0.020	oligosaccharides A and B
5	1.5%→3% ethanol	0.028	oligosaccharides B, G and arabinose
6		0.030	oligosaccharides B, C, E, F, G and arabinose
7		0.068	oligosaccharides C, D, E, F, G and arabinose
8	3→8% ethanol	0.025	oligosaccharide G and arabinose
9		0.026	oligosaccharides G, H and arabinose
10		0.032	oligosaccharides H, I and arabinose
11		0.017	oligosaccharides I and J

Examination of the fractions

Fraction 1a

Examination of this fraction (1.460 g.) by paper chromatography indicated that it was substantially composed of arabinose with a trace of xylose. The fraction was crystalline and on recrystallisation from methanol had m.p. = 150-2° and $[\alpha]_D$ = +150° → +105° (c = 2.1). From the equilibrium value of the specific rotation, the composition of this fraction would appear to be arabinose 95% and xylose 5%.

Fraction 1b

This fraction (2.039 g.) was similar to 1a but chromatographic examination showed it to contain a trace of galactose and appreciably more xylose. Again the fraction was crystalline and from a specific rotation $[\alpha]_D$ = +100° → +75° (c = 1.9) the ratio of arabinose to xylose, neglecting the very small amount of galactose, was 2:1.

Fraction 1c

Chromatographic examination of this fraction (0.120 g.) in solvents A, B and D, showed that it contained a mixture of the following monosaccharides, the relative amounts of which are indicated in parentheses: D-galactose (++), L-arabinose (+++), D-xylose (++++), D-ribose (+) and L-rhamnose (++). Further investigation of this fraction was not carried out.

Fraction 1d

This fraction (0.100 g.) was examined chromatographically in solvents A, B and D. This indicated the presence of the

following sugars in the relative amounts as shown in parentheses: L-arabinose (++), L-fucose (++++), D-xylose (++), D-ribose (+) and L-rhamnose (+). The presence of these sugars was established by comparison with authentic standards. This fraction was not examined further.

Fraction 2

While this fraction (0.030 g.) was mainly composed of arabinose, there was also a second component chromatographically indistinguishable from oligosaccharide A, which was characterised in fraction 3.

Fraction 3

Chromatographically pure oligosaccharide A [(0.023 g.), $[\alpha]_D = +180^\circ$ (c = 2.3)] was indistinguishable from 3-O-β-L-arabinopyranosyl-L-arabinose (R_{gal} = 0.85 in solvent A). Hydrolysis of a sample (2 mg.) gave arabinose as the sole component of the hydrolysate.

The oligosaccharide (5 mg.) was methylated by six additions of methyl sulphate (0.5 ml.) and 30% aqueous sodium hydroxide (1 ml.). The methylated product was methanolysed and after gas-liquid chromatographic examination of the methyl glycosides on system (C) the presence of the following sugars was indicated: 2,3,4-tri- (T = 0.83), 2,4- (T = 2.01), and 2,5-di-O-methyl-L-arabinose (T = 1.51).

The remainder of the methyl glycosides was hydrolysed and the hydrolysis products were chromatographically indistinguishable, in solvent G, from 2,3,4-tri-, 2,5- and 2,4-di-O-methyl-L-arabinose.

Fraction 4

A preliminary chromatographic examination of this fraction (0.019 g.) showed that it contained two components, and these were later separated on Whatman 3 MC filter sheet in solvent A.

Subfraction 4a

This fraction (0.004 g.) was identical to oligosaccharide A and so was not further examined.

Subfraction 4b

Chromatographically pure oligosaccharide B [(0.013 g.); $[\alpha]_D = +20^\circ$ (c = 1.3)] was indistinguishable from 6-O-β-D-galactopyranosyl-D-galactose. Hydrolysis of a sample (2 mg.) gave only galactose.

A small sample (5 mg.) was methylated by six additions of dimethyl sulphate (0.5 ml.) and sodium hydroxide (1 ml.). The methylated product was methanolysed and after gas-liquid chromatography of the methyl glycosides in system (C) evidence was obtained for the presence of the following sugars: 2,3,4,6-tetra- (T = 1.79), and 2,3,4-tri-O-methyl-D-galactose (T = 6.80).

Fraction 5

Chromatographic examination of this fraction (0.026 g.) showed it contained two components which were separated on Whatman 3 MC paper using solvent B.

Subfraction 5a

The fraction [(0.009 g.); $[\alpha]_D = +26^\circ$ (c = 0.91)] was chromatographically identical to oligosaccharide B. Hydrolysis of a sample (2 mg.) gave only galactose. The fraction was not examined further.

Subfraction 5b

This sugar (0.003 g.) was chromatographically indistinguishable from oligosaccharide G later characterised as the component of subfraction 8a. Hydrolysis of a small sample gave only arabinose.

Subfraction 5c

This fraction (0.004 g.) could not be distinguished from arabinose. A small sample (2 mg.) was methylated by the Kuhn procedure. On methanolysis and subsequent examination of the methyl glycosides by gas-liquid chromatography evidence was obtained for both 2,3,4-tri- ($\underline{T}$ = 0.85) and 2,3,5-tri-O-methyl-L-arabinose ($\underline{T}$ = 0.47 and 0.63) in system (C).

Fraction 6

Paper chromatographic examination of this fraction (0.038 g.) showed that it was composed mainly of 3-O-β-D-galactopyranosyl-L-arabinose with traces of other sugars. By thick paper chromatography in solvent B the galactosyl-arabinose was separated from the other sugars, which were discarded.

Subfraction 6a

Chromatographically pure oligosaccharide C (0.029 g.) was crystalline and indistinguishable from 3-O-β-D-galactopyranosyl-L-arabinose. Hydrolysis of a small sample (2 mg.) gave galactose and arabinose in equal amounts (visual examination of the paper chromatogram) and of the derived glycitol, only galactose.

The sugar (5 mg.) was methylated by six additions of methyl sulphate (0.5 ml.) and sodium hydroxide (1 ml.).

Methanolysis of the product followed by gas-liquid chromatography of the methyl glycosides indicated the presence of the following sugars: 2,3,4,6-tetra-O-methyl-D-galactose (T = 1.79), 2,4- (T = 2.03), and 2,5-di-O-methyl-L-arabinose (T = 1.54).

The sugar was recrystallised from aqueous ethanol. The crystals had m.p. and mixed m.p. 200-1° (with an authentic sample of 3-O-β-D-galactopyranosyl-L-arabinose melting at 202-3°) and $[\alpha]_D$ = +90° → +68° (c = 0.5, 2 hr.).

Fraction 7

A preliminary chromatographic examination of this fraction (0.068 g.) showed it to be a mixture of oligosaccharides. Separation of these was carried out by thick paper chromatography in solvent B.

Subfraction 7a

Chromatographically homogeneous, oligosaccharide D [(0.010 g.); $[\alpha]_D$ = +30° (c = 1.0)] was indistinguishable from 3-O-β-D-galactopyranosyl-D-galactose. Hydrolysis of a sample (2 mg.) gave only galactose.

Methylation of a small sample (5 mg.) by six additions of methyl sulphate (0.5 ml.) and sodium hydroxide (1 ml.) followed by methanolysis and gas-liquid chromatography of the methyl glycosides on system (C) indicated the presence of the following sugars: 2,3,4,6-tetra- (T = 1.79), and 2,4,6-tri-O-methyl-D-galactose (T = 3.70 and 4.24).

Subfraction 7b

This fraction (0.025 g.) was chromatographically identical

to oligosaccharide C. Hydrolysis gave both galactose and arabinose, and of the derived glycitol, only galactose. Further investigation of this oligosaccharide was not carried out.

Subfraction 7c

Chromatographically pure oligosaccharide E (0.005 g.) had R_{gal} = 1.50 and 1.10 in solvents A and B respectively. Hydrolysis gave only arabinose, but insufficient material prevented further investigation.

Subfraction 7d

Chromatographically homogeneous oligosaccharide F [(0.012 g.); $[\alpha]_D = +12^\circ$ (c = 1.2)] had R_{gal} = 0.95 and 0.60 in solvents A and B respectively. Hydrolysis gave mannose, xylose and rhamnose in approximately equal amounts (visual examination of paper chromatogram). Reduction to the glycitol followed by hydrolysis and chromatography in solvent I showed rhamnose, xylose and mannitol.

Subfractions 7e and 7f

These sugars whose chromatographic mobilities were identical to oligosaccharide G and arabinose were intentionally lost during the paper chromatographic separation of fraction 7, in an effort to obtain a better separation of the other components.

Fraction 8

This fraction (0.022 g.) appeared homogeneous in solvent A (R_{gal} = 1.36), but in solvent B resolved into two pink spots (R_{gal} = 1.24 and 1.71 respectively). These two components

were separated by thick paper chromatography in solvent B.

Subfraction 8a

Chromatographically pure oligosaccharide G [(0.010 g.); $[\alpha]_D$ = +100° (c = 1.0)] was indistinguishable from 3-O-β-L-arabinofuranosyl-L-arabinose in solvent A (R_{gal} = 1.37) and in solvent B (R_{gal} = 1.24). Hydrolysis of a small sample (1 mg.) gave arabinose alone.

A small scale methylation (2 mg.) by the Kuhn procedure was carried out. The product was methanolysed and after subsequent gas-liquid partition chromatography of the methyl glycosides in system (C) evidence was obtained for the presence of the following sugars: 2,3,5-tri- (T = 0.47 and 0.63), 2,5- (T = 1.51) and 2,4-di-O-methyl-L-arabinose (T = 2.01).

Subfraction 8b

This fraction (0.007 g.) was indistinguishable from arabinose in solvents A, B, C and D. A small sample (2 mg.) was methylated by the Kuhn procedure. The product was methanolysed and the methyl glycosides examined by gas-liquid chromatography in system (C). This gave evidence for the presence of both 2,3,4-, and 2,3,5-tri-O-methyl-L-arabinose.

Fraction 9

Chromatographic examination of this fraction (0.025 g.) showed it to contain oligosaccharides G, H and arabinose. It was separated into three fractions on thick paper using solvent system B.

Subfraction 9a

Chromatographically pure oligosaccharide H (0.004 g.) was

indistinguishable from an authentic sample of 1→6 galactotriose. Hydrolysis with 0.5 N-sulphuric acid for thirty minutes gave mainly galactose with a small amount of 6-O-β-D-galactopyranosyl-D-galactose (oligosaccharide B). Complete hydrolysis gave only galactose.

Subfraction 9b

This fraction (0.012 g.) was chromatographically pure and indistinguishable from oligosaccharide G. Hydrolysis gave only arabinose.

Subfraction 9c

This fraction (0.003 g.) was chromatographically indistinguishable from arabinose. Further examination was not carried out.

Fraction 10

Chromatographic examination of this fraction (0.010 g.) showed it to be composed of three sugars which were indistinguishable from oligosaccharides H and I, and arabinose. The fraction was not further examined.

Fraction 11

This fraction (0.030 g.) was separated into two components by thick paper chromatography in solvent B.

Subfraction 11a

Chromatographically pure, oligosaccharide I (0.018 g.) was indistinguishable from O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-L-arabinose. Hydrolysis gave galactose and arabinose. The sugar crystallised on desiccation and was recrystallised from aqueous ethanol, m.p. = 190-1°

(unchanged on admixture with an authentic specimen of the above oligosaccharide, melting at 190-1°).

Subfraction 11b

Chromatographically pure, oligosaccharide J (0.006 g.) had R_{gal} = 1.56 and 2.20 in solvents A and B respectively. Hydrolysis of a small sample (2 mg.) gave only arabinose.

Methylation of leiocarpan A

Leiocarpan A (18 g.) was dissolved in water (120 ml.) and treated with methyl sulphate (125 ml.) and 30% (w/v) aqueous sodium hydroxide (250 ml.) below 5° in an atmosphere of nitrogen. The reagents were added dropwise over a period of eight hours with vigorous stirring. Further similar additions were made on four successive days and secondary octyl alcohol was added occasionally to control frothing. After all additions had been completed the final mixture was heated on a boiling-water bath for one hour. After allowing to cool, the reaction mixture was dialysed against running tap water for five days, until free of sulphate ions. The partially methylated polysaccharide was isolated by concentration followed by freeze-drying (13.2 g.).

This product was dissolved in water (250 ml.) and metal ions were removed by passing through a column (2 x 30 cm.) of Amberlite resin IR 120(H^+). The silver salt of the polysaccharide was then prepared by treatment of the solution of the partly methylated gum acid with silver carbonate to pH 7, filtration, concentration of the filtrate and freeze-drying.

The silver salt (14 g.) was dissolved in the minimum of dry methanol. Methyl iodide (150 ml.) was added and the solution stirred under reflux both during the addition of the silver oxide (14 g.) in portions, and for a further eighteen hours. The reaction mixture was then filtered and the residue was continually extracted with boiling chloroform in a Soxhlet

for ten hours. The filtrate and chloroform extract were combined, dried, concentrated to small volume, and poured into a large excess (20 volumes) of light petroleum (b.p. 60-80°). The precipitated methylated polysaccharide was isolated at the centrifuge and dried in vacuo. Four additional Purdie methylations were carried out in this manner. The methoxyl content was not raised by the final methylation. The product was thus fully methylated leiocarpan A (12 g.). Found: OMe = 40.1%; $[\alpha]_D$ = +1° (c = 1.02 in $CHCl_3$).

Reduction of methylated leiocarpan A

The methylated polysaccharide (8 g.) was dissolved in tetrahydrofuran (300 ml.) and lithium aluminium hydride (8 g.) in tetrahydrofuran (300 ml.) was added slowly. The reaction mixture was stirred at room temperature for thirty minutes and then under reflux for a further three hours. Excess lithium aluminium hydride was destroyed by the addition of ethyl acetate saturated with water, and the reaction mixture was acidified (pH 4) by the addition of 2 N-sulphuric acid, whereupon the reduced polysaccharide was extracted into chloroform (5 x 100 ml.). The dried chloroform extracts were concentrated to small volume and the reduced methylated polysaccharide was precipitated by pouring into light petroleum (20 volumes). The precipitate was isolated at the centrifuge and dried in vacuo to give reduced methylated leiocarpan A (6.5 g.). Found: OMe = 37.4%; $[\alpha]_D$ = 0° (c = 1.2 in $CHCl_3$).

Hydrolysis of reduced methylated leiocarpan A

The reduced methylated polysaccharide (5.5 g.) was suspended in 2 N-hydrochloric acid (200 ml.) for three days at room temperature and then at 40-50° for a further day. The resultant solution was gradually heated to 100° over six hours, diluted to 400 ml. with water, and then heated on a boiling-water bath for ten hours (to constant rotation). The hydrolysate was neutralised with silver carbonate and the precipitated silver salts removed at the centrifuge and washed with water (4 x 100 ml.). The supernatant solution and washings were combined and treated with hydrogen sulphide gas, thus removing colloidal silver as silver sulphide which was removed by filtration through glass fibre paper. The filtrate was concentrated to a syrup (5.3 g.) which was placed on a cellulose column (3.5 x 80 cm.; 200 g. dry powder). The column was then eluted by the following solvents,

a) light petroleum (b.p. 100-120°) : butan-1-ol, (80:20, saturated with water),

b) light petroleum (b.p. 100-120°) : butan-1-ol, (70:30, saturated with water),

c) light petroleum (b.p. 100-120°) : butan-1-ol, (50:50, saturated with water),

d) butan-1-ol half saturated with water,

e) butan-1-ol saturated with water,

f) water,

g) acetone.

Fractions (15 ml.) were collected every twenty minutes on an automatic fraction collector. The contents of every third tube were concentrated and examined by paper chromatography in solvents E and F. By this method seventeen fractions were collected in all. Table XV summarizes the results of preliminary examination of the various fractions.

Table XV

Fr.	Wt. (mg.)	R_G	$[\alpha]_D$	Sugars given on de-methylation	Sugars detected in fraction	Means of detection*
1	1600	0.95-0.97	-10°	arabinose xylose	2,3,5-Me_3 arabinose 2,3,4-Me_3 xylose	F, G, g.l.c.
2	100	0.59-0.97	+44°	mannose galactose glucose arabinose xylose	2,3-Me_2 glucose† 3,4-Me_2 mannose† 3,4,6-Me_3 mannose 2,3,4,6-Me_4 galactose and other sugars	E, F, g.l.c.
4	120	0.59-0.97	+42°	xylose arabinose	2,3-Me_2 glucose† 3,4-Me_2 mannose† 2,5-Me_2 arabinose 2,3-Me_2 xylose and other sugars	E, F, I, g.l.c.
5	80	0.72-0.86	+61°	arabinose xylose	2,5-Me_2 arabinose 2,3-Me_2 xylose 2,4-Me_2 xylose and other sugars	F, G, I, g.l.c.

Table XV (contd.)

Fr.	Wt. (mg.)	R_G	$[\alpha]_D$	Sugars given on de-methylation	Sugars detected in fraction	Means of detection*
6	570	0.59-0.70	+30°	mannose glucose galactose (tr.)	3,4-Me_2 mannose 2,3-Me_2 glucose and other sugars	E, F, G, g.l.c.
7	1310	0.60	+39°	glucose mannose	2,3-Me_2 glucose 3,4-Me_2 mannose	E, F, G, g.l.c.
8	100	0.60	+64°	glucose arabinose (tr.)	2,3-Me_2 glucose 2,4-Me_2 arabinose	E, F, G, g.l.c.
9	76	0.45-0.59	+54°	glucose galactose arabinose	2,3-Me_2 glucose 2,6-Me_2 galactose 2-Me arabinose	E,F,G,I, g.l.c.
11	15	0.44	+86°		2,4-Me_2 galactose 2,6-Me_2 galactose	E, F
12	350	0.32-0.46	+29°		4-Me mannose 2-Me arabinose	E, F
13	257	0.27-0.40	+34°		4-Me mannose 3-Me glucose	E, F
14	150	0.26-0.31	+29°	mannose glucose galactose	4-Me mannose 3-Me glucose	E, F

Table XV (contd.)

Fr.	Wt. (mg.)	R_G	$[\alpha]_D$	Sugars given on de-methylation	Sugars detected in fraction	Means of detection*
15	20	0.26	+44°	glucose galactose	3-Me glucose 2-Me galactose	F
16	58		+35°	galactose mannose	2-Me galactose mannose	A, B, F
17	24				mannose arabinose	A, B

* Abbreviations used in the above and following tables (XVI-XXII) are as follows:

A, B, E, F, G = paper chromatography in solvents A, B, E, F and G respectively. I = paper ionophoresis in borate buffer.

P = paper chromatography of the periodate oxidised sugar.

g.l.c. = gas-liquid chromatography of the methyl glycosides.

tr. = trace quantity.

/ 3,4-di-O-methylmannose and 2,3-di-O-methylglucose were only detected after hydrolysis of the fraction.

Fraction 1

The observed specific rotation ($[\alpha]_D = -10°$) of this fraction showed that it was composed of an equimolar mixture of 2,3,5-tri-O-methyl-L-arabinose ($[\alpha]_D = -38°$) and 2,3,4-tri-O-methyl-D-xylose ($[\alpha]_D = +20°$).

The two components could not be satisfactorily separated by

filter sheet chromatography in solvents F and G, or in solvents employing dimethyl sulphoxide as the stationary phase, e.g. dimethyl sulphoxide (5% v/v) in benzene[87].

The sugars (0.3 g.) were oxidised to the corresponding aldonolactones. While separation of these derivatives by preparative gas-liquid chromatography did not give complete resolution, a small amount (30 mg.) of pure 2,3,4-tri-O-methyl-D-xylonolactone was isolated as a syrup which crystallised, and after recrystallisation from ether-petroleum ether, the crystals had m.p. = 49-50° (unchanged on admixture with an authentic sample, melting at 48-9°). In solvent G, however, a partial separation into two fractions was obtained. Although neither of these fractions was chromatographically pure, one was predominantly composed of 2,3,5-tri-O-methyl-arabinose, shown by its specific rotation (-33°, c = 1.4 in $CHCl_3$). Oxidation of this fraction with bromine and subsequent treatment of the product with methanolic ammonia, gave a crystalline product which after recrystallisation from ethyl acetate had m.p. and mixed m.p. = 134-5° (with an authentic specimen of 2,3,5-tri-O-methyl-L-arabonamide, m.p. = 135°).

Fractions 2 and 3

A preliminary examination of fractions 2 and 3 indicated that they were composed of similar mixtures of sugars, and they were thus combined. Chromatographic examination of the fraction indicated the presence of several components, and after hydrolysis 2,3-di-O-methylglucose and 3,4-di-O-methyl-

mannose were detected as additional constituents.

The fraction (0.095 g.) was hydrolysed and the hydrolysate was chromatographed on a charcoal-Celite column (80 g., 3 x 35 cm.) by gradient elution with water containing 0→10% of ethanol (5 l.) followed by continued elution with water containing 2% of butan-2-one, until all sugars had been desorbed. Subfractions 2a - 2c were obtained by elution with the ethanol solution, while 2d was eluted with the solution of butan-2-one. Fractions 2a and 2d were then further fractionated by thick paper chromatography in solvent F. Results from preliminary examination of the subfractions are given below (Table XVI).

Table XVI

Fraction		Wt. (mg.)	Sugars given on demethylation	Sugars detected in fraction	Means of detection
2a		30		2,3-Me_2 glucose 3,4-Me_2 mannose 2,4-Me_2 xylose	F, g.l.c.
	$2a_1$	10	glucose mannose	2,3-Me_2 glucose 3,4-Me_2 mannose	F, I, g.l.c.
	$2a_2$	6	xylose	2,3-Me_2 xylose 2,4-Me_2 xylose	F, g.l.c.
2b		9		3,4,6-Me_3 mannose	F, g.l.c.
2c		10	mannose arabinose	3,4,6-Me_3 mannose 2,5-Me_2 arabinose	F, G, I, g.l.c.
2d		24	galactose and other sugars	2,3,4,6-Me_4 galactose 2,3,4-Me_3 glucose	F, g.l.c.

Table XVI (contd.)

Fraction	Wt. (mg.)	Sugars given on demethylation	Sugars detected in fraction	Means of detection
$2d_1$	10	glucose arabinose galactose (tr.)	2,3,4-Me_3 glucose 2,3,4,6-Me_4 galactose 2,5-Me_2 arabinose	P, G, g.l.c.
$2d_2$	6		2,3,4,6-Me_4 galactose	g.l.c.
$2d_3$	3		2,3,5-Me_3 arabinose 2,3,4-Me_3 xylose	P, G, g.l.c.

Subfraction 2b

The syrup crystallised on standing and was recrystallised from ether, m.p. = 104-5° (not depressed on admixture with authentic 3,4,6-tri-O-methyl-D-mannose, m.p. = 106°).

Subfraction $2d_2$

The sugar was characterised by conversion into the aniline derivative, which, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 195-6° (with an authentic specimen of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine, m.p. = 197°).

Fraction 4

Like the previous fraction, this fraction (0.120 g.) contained a number of sugars and after hydrolysis gave both 2,3-di-O-methylglucose and 3,4-di-O-methylmannose as additional sugars. After hydrolysis the fraction was further fractionated on a charcoal-Celite column (55 g., 2.5 x 30 cm.) by gradient elution with water containing 0→8% of ethanol (6 l.) to give fractions 4a - 4d, fraction 4a being further fractionated by filter sheet chromatography in solvent F. The results of preliminary investigations on these fractions is shown below (Table XVII).

Table XVII

Fraction		Wt. (mg.)	Sugars given on demethylation	Sugars detected in fraction	Means of detection
4a		50		2,3-Me_2 xylose 2,5-Me_2 arabinose	E, F, G, g.l.c.
	$4a_1$	15		2,5-Me_2 arabinose	F, g.l.c.
	$4a_2$	8		2,5-Me_2 arabinose 2,3-Me_2 xylose	F, g.l.c.
	$4a_3$	6	xylose	2,3-Me_2 xylose	F, g.l.c.
	$4a_4$	6		2,3,4-Me_3 xylose 2,3,5-Me_3 arabinose	g.l.c.
4b		11	glucose mannose xylose arabinose (tr.)	2,3-Me_2 glucose 3,4-Me_2 mannose 3,4-Me_2 xylose 3,5-Me_2 arabinose	F, I, g.l.c.
4c		4	arabinose	2,3,4-Me_3 arabinose	F, G, g.l.c.
4d		11	arabinose mannose	2,3,4-Me_3 arabinose 3,4,6-Me_3 mannose	F, G, g.l.c.

Subfraction $4a_1$

The sugar was characterised by conversion to the aldonolactone and then to the aldonamide which was crystalline,

m.p. = 126-7° (unchanged on admixture with an authentic sample of 2,5-di-O-methyl-L-arabonamide, m.p. = 129-30°).

Subfraction 4b

Examination of this fraction by borate electrophoresis showed the presence of three mobile components. By comparison with authentic standard sugars, two of these were indistinguishable from 3,4-di-O-methylmannose and 3,4-di-O-methylxylose. The third component (M_g = 0.68) was possibly 3,5-di-O-methylarabinose since there was a trace of arabinose after demethylation of the fraction. Examination of the fraction by gas-liquid chromatography showed that 2,3,4-tri-O-methylarabinose was absent.

Fraction 5

Further fractionation of this fraction on a charcoal-Celite column (55 g., 2.5 x 30 cm.) by gradient elution with water containing 0 → 8% of ethanol (6 l.) gave four sub-fractions. Preliminary examination of these fractions gave the results indicated below (Table XVIII).

Table XVIII

Fraction	Wt. (mg.)	Sugars given on demethylation	Sugars detected in fraction	Means of detection
5a	15	xylose arabinose (tr.)	2,3-Me_2 xylose 2,4-Me_2 xylose 2,5-Me_2 arabinose	F, I, g.l.c.
5b	11		2,3-Me_2 xylose 2,4-Me_2 xylose	g.l.c., F
5c	8		2,3,4-Me_3 arabinose 2,4,6-Me_3 galactose	F, G, g.l.c.
5d	3		2,3,4-Me_3 arabinose	G, g.l.c.

Fraction 6

On standing one of the components of this fraction (0.570 g.) crystallised out. The crystals were removed by filtration and washed with cold ethyl acetate which was then added to the mother liquor. The crystalline sugar (0.2 g.) was recrystallised from ethyl acetate, m.p. = 112-4° (unchanged on admixture with an authentic sample of 3,4-di-O-methyl-D-mannose monohydrate, m.p. = 110-1°). The sugar (0.05 g.) was further characterised by conversion into 3,4-di-O-methyl-D-mannonolactone, m.p. and mixed m.p. = 157-8° (with an authentic specimen melting at 159-60°).

The remainder of this fraction (0.370 g.) was further fractionated on a charcoal-Celite column (55 g., 2.5 x 30 cm.)

by gradient elution with water containing $0 \rightarrow 5\%$ of ethanol (5 l.), followed by continued elution with a 6% solution of ethanol in water until all sugars had been desorbed. No further sugars were eluted with water containing 15% of butan-2-one. By this method seven fractions, 6a - 6g, were obtained and of these 6a, 6c, 6d and 6f were further fractionated by filter sheet chromatography in solvent F. Preliminary results of investigations on these subfractions are given below (Table XIX).

Table XIX

Fraction		Wt. (mg.)	Sugars given on de-methylation	Sugars detected in fraction	Means of detection
6a		40		2,4-Me_2 arabinose 2,3-Me_2 arabinose	F, g.l.c.
	$6a_1$	4	arabinose	2,4-Me_2 arabinose	F, G, g.l.c.
	$6a_2$	26		2,3-Me_2 arabinose	F, g.l.c.
6b		7		2,3-Me_2 arabinose	g.l.c.
6c		100		2,3-Me_2 glucose 2,3-Me_2 arabinose	g.l.c.
	$6c_1$	66	glucose	2,3-Me_2 glucose	F, g.l.c.
	$6c_2$.	7		2,3-Me_2 arabinose	F

Table XIX (contd.)

Fraction		Wt. (mg.)	Sugars given on de-methylation	Sugars detected in fraction	Means of detection
6d		93		2,3-Me_2 glucose 3,4-Me_2 mannose 2,4-Me_2 xylose	F, g.l.c.
	$6d_1$	72	glucose mannose	2,3-Me_2 glucose 3,4-Me_2 mannose	g.l.c.
	$6d_2$	12	xylose	2,4-Me_2 xylose	F, g.l.c.
6e		32		3,4-Me_2 mannose	F
6f		31		3,4-Me_2 mannose 2,4,6-Me_3 galactose	F, g.l.c.
	$6f_1$	20		3,4-Me_2 mannose	g.l.c.
	$6f_2$	4		2,4,6-Me_3 galactose	g.l.c.
6g		26		2,4,6-Me_3 galactose	g.l.c.

Subfraction $6a_2$

The sugar was characterised as 2,3-di-O-methyl-L-arabinose by conversion into 2,3-di-O-methyl-L-arabonamide, which, after recrystallisation from ethyl acetate, had m.p. and mixed m.p. = 150-1° (with an authentic sample, m.p. = 154°).

Subfraction $6d_2$

The sugar, suspected to be 2,4-di-O-methyl-D-xylose,

crystallised on desiccation, m.p. = 107-8°, but attempts to re-crystallise the sugar from ethyl acetate were unsuccessful.

Subfraction 6e

The sugar crystallised on standing, m.p. = 112-3° (not depressed on admixture with an authentic sample of 3,4-di-O-methyl-D-mannose monohydrate, m.p. = 112°).

Subfraction 6g

The sugar was characterised as 2,4,6-tri-O-methyl-D-galactose by conversion into the aniline derivative which had m.p. = 169-70° (unchanged on admixture with an authentic specimen, m.p. = 168-9°).

Fraction 7

The syrup (1 g.) was fractionated on a charcoal-Celite column (200 g., 5 x 27 cm.) by gradient elution with water containing 0 → 5% of ethanol (8 l.), followed by continued elution with a solution of 5% of ethanol in water until all the 3,4-di-O-methylmannose had been desorbed. By this method three fractions were obtained.

Subfraction 7a

This fraction (0.106 g.) was further fractionated by filter sheet chromatography in solvent G to give 2,4-di-O-methylarabinose (0.012 g.) and 2,3-di-O-methylglucose (0.07 g.). Characterisation of the 2,4-di-O-methylarabinose as its aniline derivative failed.

Subfraction 7b

Chromatographically pure 2,3-di-O-methyl-D-glucose

(0.48 g.) crystallised after desiccation for three months, and had m.p. = 98-100°; $[\alpha]_D$ = +25° → +71° (9 hr., c = 1.05). The sugar was further characterised by conversion into the aniline derivative, which after recrystallisation from ethyl acetate had m.p. and mixed m.p. = 131-2° (with an authentic specimen of 2,3-di-O-methyl-N-phenyl-D-glucosylamine, m.p. = 130-1°).

Subfraction 7c

This fraction (0.310 g.) was chromatographically indistinguishable from 3,4-di-O-methylmannose. The sugar crystallised and after recrystallisation from ethyl acetate had m.p. = 112-3° (not depressed on admixture with authentic 3,4-di-O-methyl-D-mannose monohydrate, m.p. = 112°).

Fractions 9 and 10

Preliminary investigation of fractions 9 and 10 showed that they were composed of similar mixtures of sugars, and they were thus combined. The fraction was further fractionated by filter sheet chromatography in solvent F to give three subfractions 9a - 9c. Subfractionation of 9b was achieved on a charcoal-Celite column (50 g., 25 x 2.5 cm.) by gradient elution with water containing 0→4% ethanol (3 l.) which gave subfraction $9b_1$. Further elution of the column with a solution of water containing 15% of butan-2-one gave subfraction $9b_2$. A preliminary examination of the subfractions gave the following results (Table XX).

Table XX

Fraction		Wt. (mg.)	Sugars given on de-methylation	Sugars detected in fraction	Means of detection
9a		7	galactose	2,4-Me_2 galactose	E, F, G
9b		34		2-Me arabinose 2,6-Me_2 galactose	E, F, I, g.l.c.
	$9b_1$	18	arabinose	2-Me arabinose	F, I, g.l.c.
	$9b_2$	9	galactose	2,6-Me_2 galactose	F, I, g.l.c.
9c		11		2,3-Me_2 glucose	F, g.l.c.

Subfraction $9b_1$

This sugar, suspected to be 2-O-methylarabinose[88], on treatment with toluene-p-sulphonyl hydrazine gave a product which was partly crystalline. Attempts to recrystallise the crystals (m.p. 134-9°) from methanol, were unsuccessful.

Fraction 11

Fractionation of this fraction by filter sheet chromatography in solvent F gave two subfractions.

Subfraction 11a

The sugar (0.004 g.) was chromatographically indistinguishable from 2,4-di-O-methylgalactose.

Subfraction 11b

This fraction (0.008 g.) which contained the same components as 9b, was not further examined.

Fraction 12

Chromatographic separation of this fraction (0.350 g.) on filter sheets in solvent F gave subfractions 12a and 12b of which 12a was further fractionated on a charcoal-Celite column (55 g., 30 x 2.5 cm.) by gradient elution with water containing $0 \rightarrow 2\%$ of ethanol (4 l.). Both components were desorbed by this range of concentration. Preliminary examination of the fractions gave the following results (Table XXI).

Table XXI

Fraction		Wt. (mg.)	Sugars given on demethylation	Sugars detected in fraction	Means of detection
12a		270	mannose galactose	4-Me mannose 2,4-Me_2 galactose	E, F
	$12a_1$	220		4-Me mannose	E, F, P
	$12a_2$	25		2,4-Me_2 galactose	E, F
12b		15	arabinose	2-Me arabinose	E, F, I

Subfraction $12a_1$

The sugar (0.270 g.) crystallised on standing to give 4-O-methyl-D-mannose, and was recrystallised from ethanol, $[\alpha]_D = +30^\circ \rightarrow +22^\circ$ (10 hr., c = 1.2), m.p. = 128-9° (unchanged on admixture with an authentic specimen of 4-O-methyl-D-mannose, m.p. = 127-8°). The sugar (0.02 g.) was further characterised by conversion into 4-O-methyl-D-mannonolactone, m.p. = 163-4° (not depressed on admixture with an authentic specimen, m.p. = 164-5°).

Subfraction $12a_2$

The sugar, which crystallised on standing for six weeks, was recrystallised from acetone containing 1% of water, to give 2,4-di-O-methyl-D-galactose, m.p. and mixed m.p. = 99-100° (not depressed on admixture with an authentic sample, m.p. 100-1°).

Fractions 13, 14 and 16

These were further fractionated by filter sheet chromatography in solvent F. The results of preliminary examination of the subfractions are tabulated below (Table XXII).

Table XXII

Fraction	Wt. (mg.)	Sugars given on demethylation	Sugars detected in fraction	Means of detection
13a	20		3-Me glucose	F, P, I
13b	170	mannose galactose	4-Me mannose 2,4-Me_2 galactose	F
14a	20	glucose galactose	2-Me galactose 3-Me glucose	F, P, I
14b	51		4-Me mannose	F
16a	15		mannose	A, B
16b	20		2-Me galactose	A, F, P

Subfraction 13a

The sugar crystallised on standing and was recrystallised

from acetone-water to give 3-O-methyl-D-glucose, m.p. = 152-3° (unchanged on admixture with an authentic specimen, m.p. = 150-1°).

Subfraction 14b

This fraction was crystalline and after recrystallisation from ethanol gave 2-O-methyl-D-galactose, m.p. 127-8° (unchanged on admixture with an authentic specimen, m.p. = 129-30°).

Preparation of carboxyl reduced leiocarpan A

a. Formation of the glycol ester

Leiocarpan A (34 g.) in water (1700 ml.) was passed through a column (3 x 30 cm.) of Amberlite resin IR 120(H^+) to effect complete removal of cations. To the de-ionised solution (pH 2.5) ethylene oxide (300 ml.) was added and the solution, in a stoppered flask, was allowed to stand at room temperature. Over the first four days the pH of the solution rose to 4.6 at which it remained until further additions of ethylene oxide (2 x 200 ml.) raised it to 6.4 and 6.8 respectively. Further addition of ethylene oxide (100 ml.) failed to affect the pH of the solution. Removal of excess ethylene oxide was achieved by reduced pressure evaporation and after concentration (ca. 500 ml.) the solution was dialysed against tap water for five days. The non-diffusate was concentrated (200 ml.) and freeze-dried to give leiocarpan A glycol ester (36 g.).

b. Formation of the glycol ester acetate

The glycol ester of leiocarpan A (36 g.) was divided into three equal portions which were acetylated individually as follows.

The glycol ester (12 g.) was dissolved in formamide (300 ml.) and the solution stirred at 30° for four hours. The temperature of the solution was raised to 45° and pyridine (400 ml.) freshly redistilled over potassium hydroxide was

added dropwise over two hours. After cooling the solution again to 30° acetic anhydride (320 ml.) was added dropwise over two hours. Stirring at 30° was then continued for a further two hours and then for eighteen hours at room temperature. Care was taken that anhydrous conditions were maintained as far as this stage of the reaction sequence. The reaction mixture was finally poured, dropwise, with vigorous stirring, into ice cold hydrochloric acid (6 l.; 2% w/v).

The precipitated glycol ester acetate was removed at the centrifuge and washed with water (4 x 2 l.). The acetate was then dissolved in acetone and water removed by azeotropic distillation with chloroform. By this process the acetate was eventually chloroform soluble, and concentration of the dried chloroform solution (ca. 50 ml.) and precipitation in light petroleum (40-60°; 1 l.), gave the acetate as a white powder which was removed by filtration and dried in vacuo (15.1 g.).

The two further batches of glycol ester (each 12 g.) were acetylated by an identical procedure. Yields = 14.8 g. and 15.4 g. respectively.

c. Reduction of the glycol ester acetate

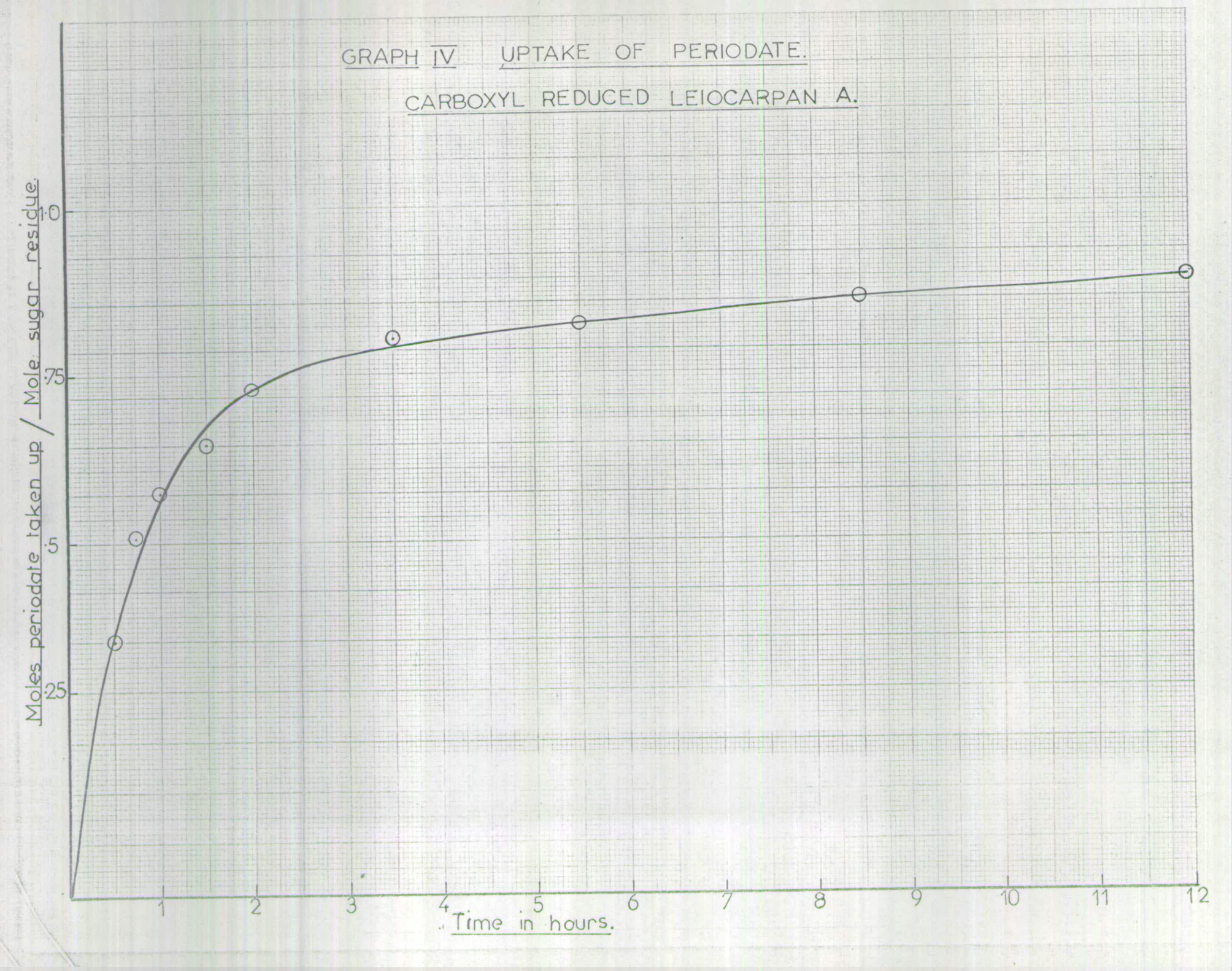
The batches of acetate prepared as above, (b), were reduced independently as follows.

Glycol ester acetate (15.1 g.) was dissolved in tetrahydrofuran (250 ml.). Lithium borohydride (15.1 g.) in tetrahydrofuran (250 ml.) was carefully added to the acetate solution. On the addition of the lithium borohydride the

acetate was immediately precipitated out of solution. The reaction mixture was then stirred at room temperature for two hours and then under reflux for eighteen hours. Destruction of lithium borohydride was achieved by the very careful addition of water. The mixture was then acidified (pH 4) by the addition of 2 N-sulphuric acid, dialysed against tap water for five days, concentrated (200 ml.) and freeze-dried to give carboxyl reduced leiocarpan A (8.4 g.).

The remaining two batches of acetate (15.3 g. and 14.8 g.) were reduced by an identical method. Yields = 8.3 g. and 7.4 g. respectively.

The carboxyl reduced polysaccharide (24.1 g.), had a uronic acid anhydride content of 2.2% (by decarboxylation) and 0.9% (by carbazole estimation), $[\alpha]_D = +37^\circ$ (c = 1.7). Hydrolysis of a small sample (10 mg.) with N-sulphuric acid at 100° for four hours gave the following monosaccharides in the relative amounts shown in parentheses: D-galactose (+), D-glucose (++++), D-mannose (+++), L-arabinose (+++), D-xylose (+++), L-rhamnose (trace) and unknown sugar (trace). The unknown component (R_{gal} = 1.74) was still present after treatment of the hydrolysate with a 5% solution of ammonia in water for two hours at room temperature. This sugar would not therefore appear to be a product of incomplete de-acetylation.


GRAPH IV UPTAKE OF PERIODATE.
CARBOXYL REDUCED LEIOCARPAN A.
Moles periodate taken up / Mole sugar residue.
1·0
·75
·5
·25
1
2
3
4
5
6
7
8
9
10
11
12
Time in hours.

Smith degradation of reduced leiocarpan A

Carboxyl reduced leiocarpan A (14 g.) was oxidised by sodium metaperiodate (25 g.) in water (2 l.). The uptake of periodate was measured spectrophotometrically and the rate of uptake is shown on Graph IV. Over the first five hours approximately 0.8 moles of periodate were consumed for every mole of sugar residue and thereafter a slow steady consumption of periodate was observed (Graph IV). The reaction was stopped after twenty-five hours by the addition of a quantity of ethylene glycol calculated to be only slightly in excess of the amount required for total destruction of the remaining periodate. Low molecular weight material was removed by dialysis against running tap water and after concentration (500 ml.) reduction to the polyalcohol was effected by the addition of sodium borohydride (3 g.) in portions over two days. Destruction of excess borohydride and removal of cations was effected with Amberlite resin IR 120(H^+). Further treatment with the same resin and several evaporations with methanol effected removal of inorganic ions.

The polyalcohol (12.6 g.), isolated as a dried syrup, was dissolved in, and hydrolysed with 0.3 N-sulphuric acid at room temperature for one and a half hours. (From a preliminary experiment it was shown that the specific rotation of the polyalcohol in the sulphuric acid changed from $+48^{\circ} \longrightarrow +32^{\circ}$ over the first ninety minutes and then remained steady (extended hydrolysis resulted in cleavage of glycosidic linkages).) After neutralisation, examination of the syrup by paper

chromatography indicated the presence of glycerol and chromatographically immobile material (degraded gum B).

The hydrolysate was initially fractionated on a column (2.2 x 110 cm.) of Dowex resin, 50 W x 8 (mesh 200-400) which had been prepared in the barium form. The sugars were eluted with water, fractions (3 ml.) being collected every ten minutes. The contents of every third tube were concentrated and examined chromatographically in solvent A. Similar fractions were combined and concentrated to give dried syrups.

Fraction 1

This fraction (0.669 g.) was chromatographically immobile in solvent A and appeared as a short streak at the starting line on the chromatogram. This fraction was obviously composed of a number of high oligosaccharides and was named degraded gum B. It was investigated by partial hydrolysis, methylation and periodate oxidation studies.

Fraction 2

This fraction (0.390 g.) contained a further amount of degraded gum B as well as an oligosaccharide which was chromatographically mobile (R_{gal} = 1.08). This oligosaccharide was separated from degraded gum B by partition chromatography on filter sheets in solvent A. The sugar had $[\alpha]_D = +68^\circ$ ($\underline{c}$ = 1.28) and on hydrolysis gave mannose and erythritol in approximately equal proportions.

A sample (5 mg.) of the sugar was methylated by the Kuhn procedure. The product was methanolised, the cleavage products were examined by gas-liquid chromatography on column (c), and by

this means methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose ($\underline{T}$ = 1.41) and 1,3,4-tri-O-methyl-D-erythritol ($\underline{T}$ = 0.31) were detected.

A sample of the sugar was examined by periodate oxidation.

a) Formaldehyde release

A solution (5 ml.) of the suspected mannosyl-erythritol in water (5 mg./ml. determined by the phenol-sulphuric acid method) was diluted to 25 ml. by the addition of 0.05 M-sodium meta-periodate solution (20 ml.) and kept at 2° in the dark. Samples (1 ml.) were withdrawn every fifteen minutes and the amount of formaldehyde released was estimated by the chromotropic acid method[92]. A constant value of 0.93 mole/mole of sugar residue was obtained for the release of formaldehyde.

b) Uptake of periodate and release of formic acid

A second solution (5 ml.) of mannosyl-erythritol (5 mg./ml.) was diluted to 25 ml. by the addition of 0.05 M-sodium meta-periodate solution (20 ml.) and kept at 2° in the dark. The consumption of periodate was measured spectrophotometrically. A rapid uptake of 2.5 mole/mole of sugar occurred over the first half hour and after twelve hours the uptake was 2.86 mole periodate/mole of sugar. Excess of periodate was then removed by the addition of ethylene glycol (1 ml.) and the solution kept for half an hour before titration with 0.0102 N-barium hydroxide solution to pH 5. From this it was shown that there was a release of 0.9 mole of formic acid per mole of sugar.

Fraction 3

This fraction (1.307 g.) contained a number of components.

A sample (5 mg.) of the fraction was dissolved in water and reduced with sodium borohydride. Hydrolysis of the reduced products with N-sulphuric acid at 100° for four hours and examination of the hydrolysate by paper chromatography in solvents A and B failed to detect any reducing sugars. It was thus apparent that this fraction did not contain any glycosidically linked sugars, and so was discarded.

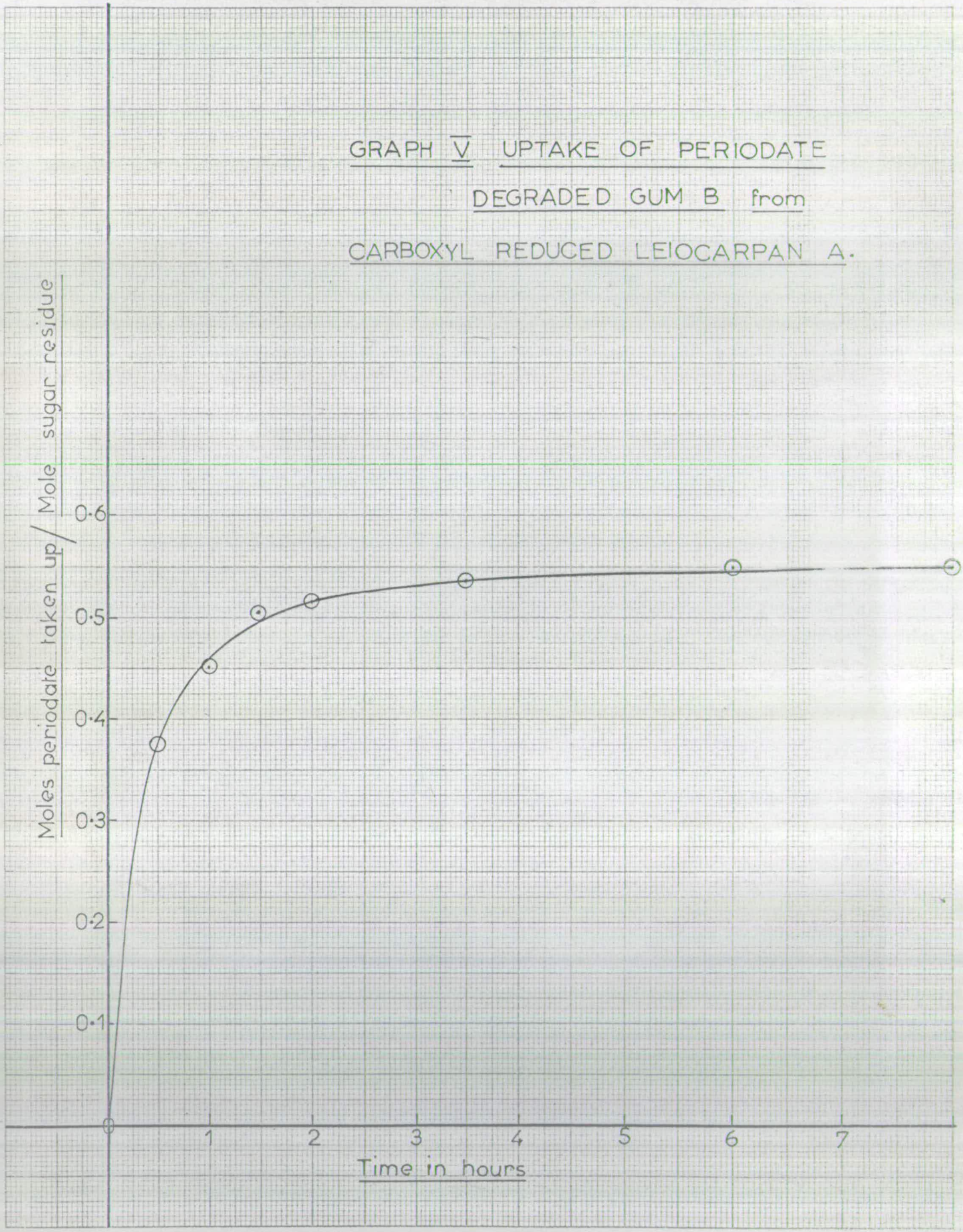
Degraded gum B

Degraded gum B had $[\alpha]_D$ = +28.4° (c = 1.90). Hydrolysis gave galactose, arabinose, mannose and erythritol. Partial hydrolysis with 0.5 N-sulphuric acid at 100° for one hour gave a number of oligosaccharides amongst which the following were recognized by comparison of their chromatographic mobility with authentic samples in solvents A, B and C, O-β-D-galactosyl-(1→6)-O-β-D-galactosyl-(1→3)-L-arabinose, 3-O-β-D-galactosyl-L-arabinose, 3-O-β-D-galactosyl-D-galactose and 6-O-β-D-galactosyl-D-galactose.

A small sample of the degraded gum B was methylated by the Kuhn procedure. The product was methanolysed and after gas-liquid chromatography of the cleavage products, methyl glycosides of a large number of methylated sugars were detected and these are listed below (Table XXIV).

The remainder of the methyl glycosides was hydrolysed with N-sulphuric acid at 100° for three hours, and the hydrolysate examined by paper chromatography in solvent F. In addition to the sugars detected as methyl glycosides, both 2,4-di- and 2-O-methyl-D-galactose were recognized.

GRAPH V UPTAKE OF PERIODATE DEGRADED GUM B from CARBOXYL REDUCED LEIOCARPAN A.



Smith degradation of degraded gum B

Degraded gum B (0.610 g.) was oxidised with sodium meta-periodate (1.5 g.) in water (150 ml.). The uptake of periodate was again measured spectrophotometrically and during the first three hours approximately 0.5 moles of periodate were consumed for every mole of sugar present and thereafter a very small uptake of periodate was observed (see Graph V). After twenty-four hours the reaction was stopped by destruction of the excess periodate by the addition of an amount of ethylene glycol calculated to be only slightly in excess of the amount required for total destruction of the remaining periodate. Sodium ions were then removed by passing through a column of Amberlite resin IR 120(H^+) and the iodic acid was then removed by neutralisation with barium carbonate. After centrifugation, cations, present in the centrifugate, were removed, and the solution was concentrated (50 ml.) and was treated with sodium borohydride (100 mg.) added over ten hours. The solution was allowed to stand overnight after which excess borohydride and sodium ions were removed by treatment with Amberlite resin IR 120(H^+). Further treatment with the same resin and several evaporations with methanol effected removal of inorganic ions.

The polyalcohol so formed (0.500 g.) was hydrolysed with 0.5 N-sulphuric acid in the cold for two hours. The hydrolysate was fractionated, as above, by chromatography on Dowex resin. After bulking of tubes, two main fractions were obtained.

The first of these was chromatographically immobile in

solvent A and was named degraded gum C, $[\alpha]_D$ = +22° (c = 1.1). The second fraction (0.223 g.) contained a number of components of which glycerol was predominant. A sample (5 mg.) of this fraction in water was reduced with sodium borohydride and hydrolysed as above. Again reducing sugars were not obtained and so this fraction was discarded.

Degraded gum C on hydrolysis with N-sulphuric acid at 100° for four hours gave galactose, arabinose, mannose and glycerol. Partial hydrolysis with 0.5 N-sulphuric acid at 100° for one hour gave the disaccharides 3-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-galactopyranosyl-L-arabinose and 6-O-β-D-galactopyranosyl-D-galactose. A sample of degraded gum C (10 mg.) was methylated by the Kuhn procedure and the product was methanolysed. Examination of the cleavage products by gas-liquid chromatography on a number of different columns indicated the presence of methyl glycosides of those sugars which are listed below (Table XXIV). The remainder of the methyl glycosides was hydrolysed with N-sulphuric acid at 100° for three hours. The hydrolysate was examined by paper chromatography in solvent F and 2,4,6-tri- and 2,4-di-O-methyl-D-galactose were detected along with a trace of the 2-monomethyl ether.

Smith degradation of degraded gum C

Degraded gum C (0.120 g.) was oxidised by sodium meta-periodate (0.25 g.) in water (25 ml.). The reaction, having reached completion in two hours, was stopped after one day. The Smith degradation was completed by an identical method to

that employed in the preparation of degraded gum C. Hydrolysis of the polyalcohol and fractionation of the hydrolysate gave degraded gum D and a number of chromatographically mobile components which were discarded.

Hydrolysis of degraded gum D, $[\alpha]_D = +20^\circ$ (c = 0.9) gave galactose, arabinose, mannose and glycerol. A partial hydrolysis with 0.5 N-sulphuric acid at 100° for one hour gave, in addition to the above monosaccharides, 3-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-galactopyranosyl-L-arabinose and a third oligosaccharide whose chromatographic mobility (R_{gal} = 0.81) was identical to that of 3-O-L-arabinopyranosyl-D-mannose, prepared from a similar partial hydrolysis of twice Smith degraded gum ghatti.

A small sample of degraded gum D (5 mg.) was methylated by the Kuhn procedure and the product was methanolysed. The cleavage products were examined by gas-liquid chromatography, and methyl glycosides of a number of sugars were detected (Table XXIV).

Table XXIV

Methylated sugar	Degraded gum B	Degraded gum C	Degraded gum D
2,3,5-tri-O-methyl-L-arabinose[Ø]	+++	++	trace
2,5-di-O-methyl-L-arabinose[Ø]	++	trace	-
2,3-di-O-methyl-L-arabinose[Ø]	+	n.d.	-
2-mono-O-methyl-L-arabinose[Ø*]	trace	-	-
2,3,4-tri-O-methyl-L-arabinose[Ø*]	trace	?(trace)	trace
2,4-di-O-methyl-L-arabinose[Ø]	++	++	++
2,3,4,6-tetra-O-methyl-D-mannose[Ø]	(++)	n.d.	-
2,4,6-tri-O-methyl-D-mannose[Ø]	++	++	++
2,3,4,6-tetra-O-methyl-D-galactose[Ø]	trace	+	+
2,4,6-tri-O-methyl-D-galactose[Ø*]	trace	+	+
2,4-di-O-methyl-D-galactose[*]	+	trace	-
2-mono-O-methyl-D-galactose[*]	++	trace	-
1,3,4-tri-O-methyl-D-erythritol[Ø]	+	n.d.	-

[*] - detected by paper chromatography of the hydrolysate.

[Ø] - detected by gas-liquid chromatography of the methyl glycosides.

n.d. - not detected.

Smith degradation of degraded gum D

Degraded gum D (0.024 g.; estimated by the phenol-sulphuric acid reagent, using galactose as standard) was oxidised by sodium metaperiodate (0.03 g.) in water (10 ml.). The consumption of oxidant was measured spectrophotometrically

and it was found that over a six hour period, 0.28 moles of periodate were consumed for every mole of sugar residue. After completion of the Smith degradation in the same way as for the preparation of degraded gums C and D, the polyalcohol was hydrolysed and the hydrolysate was fractionated by filter sheet chromatography in solvent A. The least mobile component (0.014 g.) was hydrolysed with 0.5 N-sulphuric acid for half an hour at 100°. The hydrolysate was examined by paper chromatography against authentic standard sugars and in addition to monosaccharides, the oligosaccharides 3-O-β-D-galactopyranosyl-L-arabinose, 3-O-L-arabinopyranosyl-D-mannose and a trace of 3-O-β-D-galactopyranosyl-D-galactose were detected.

Methylation of suspected O-(β-D-glucopyranosyluronic acid)-(1→2)-O-α-D-mannopyranosyl-(1→4)-O-(β-D-glucopyranosyluronic acid)-(1→2)-D-mannose from the partial acid hydrolysis of leiocarpan A.

The oligosaccharide (0.3 g.) was dissolved in water (10 ml.) and treated with methyl sulphate (8 ml.) and 30% (w/v) aqueous sodium hydroxide (16 ml.) in an atmosphere of nitrogen. The reagents were added dropwise, over a period of four hours with vigorous stirring, and four similar additions were made on the four successive days. The final mixture was heated on a boiling-water bath for one hour, thus destroying all remaining methyl sulphate. After cooling, the reaction mixture was acidified (pH 4) by the addition of 2 N-sulphuric acid, and the bulk of the sodium sulphate precipitated by pouring the aqueous solution (250 ml.) into methanol (2 l.). The precipitated sodium sulphate was removed at the centrifuge and the centrifugate was concentrated (20 ml.) and extracted with chloroform (5 x 100 ml.).

The dried chloroform extracts were concentrated to give the partly methylated product (0.150 g.) which was dissolved in methyl iodide (5 ml.), and silver oxide (0.100 g.) was added over four hours. The reaction mixture was maintained under reflux both during and for eighteen hours after the addition of silver oxide. The mixture was filtered and the residue extracted with boiling chloroform in a Soxhlet extractor. The filtrate and chloroform extracts were combined, dried and

concentrated to give a syrup. Three such Purdie methylations were carried out, the last of which failed to raise the methoxyl content of the methylated product (0.140 g.). Found: OMe = 48%; $[\alpha]_D = -18^\circ$ (c = 1.4 in $CHCl_3$).

A small sample (5 mg.) of the product was methanolised and, after gas-liquid chromatography of the methyl glycosides in system (C), evidence was obtained for the presence of the following sugars: 3,4,6-tri-O-methyl-D-mannose (T = 2.76), 2,3,4-tri- (T = 3.12, 2.37) and 2,3-di-O-methyl-D-glucuronic acid (T = 7.24, 8.0).

The remainder of the methanolysate was hydrolysed with N-sulphuric acid at 100° for four hours. The hydrolysate was adsorbed on a small DEAE-sephadex column (8 x 1 cm.) which had been prepared in the formate form. Elution of the neutral sugars with water, and concentration gave a syrup which on chromatographic examination in solvents E and F gave evidence for the presence of a tri-O-methylmannose (probably 3,4,6-) and showed that no di-O-methylmannose was present.

The remainder of the methylated oligosaccharide (0.130 g.) was dissolved in tetrahydrofuran (5 ml.) and lithium aluminium hydride (0.130 g.) in tetrahydrofuran (5 ml.) was added. The reaction mixture was stirred at room temperature for thirty minutes and then under reflux for a further three hours. Excess lithium aluminium hydride was destroyed by the addition of ethyl acetate saturated with water and the solution acidified (pH 4) by the addition of 2 N-sulphuric acid, was extracted with chloroform (5 x 100 ml.). The dried chloroform

extracts were combined and concentrated to a syrup (0.085 g.). Found: OMe 44%; $[\alpha]_D = -20^\circ$ (c = 0.94 in $CHCl_3$).

A small sample (5 mg.) was methanolised and the products examined by gas-liquid chromatography in system (C). This gave evidence for the presence of the following sugars: 3,4,6-tri-O-methyl-D-mannose (T = 2.74), 2,3,4-tri- (T = 2.31, 3.26) and 2,3-di-O-methyl-D-glucose (T = 9.18, 12.56).

The remainder of the reduced methylated oligosaccharide was hydrolysed with N-sulphuric acid at 100° for six hours.

A preliminary chromatographic examination of the hydrolysate showed that it was composed of three sugars and these were fractionated by filter sheet chromatography in solvent F.

Fraction 1

This sugar (0.013 g.) was chromatographically pure and identical to 2,3-di-O-methylglucose. The sugar was characterised by conversion into the aniline derivative, which, after recrystallisation from ethyl acetate-ether, had m.p. = 131-2° (not depressed on admixture with an authentic sample of 2,3-di-O-methyl-N-phenyl-D-glucosylamine, m.p. = 133°).

Fraction 2

This fraction (0.014 g.) was chromatographically indistinguishable from 3,4,6-tri-O-methylmannose. The sugar crystallised on standing and was recrystallised from ether, m.p. = 104-6° (unchanged on admixture with an authentic sample of 3,4,6-tri-O-methyl-D-mannose, m.p. = 106°).

Fraction 3

Chromatographic examination of this fraction (0.015 g.) showed that it contained both 3,4,6-tri-O-methylmannose and 2,3,4-tri-O-methylglucose. Further examination was not carried out.

Fraction 4

The sugar (0.009 g.) was chromatographically indistinguishable from 2,3,4-tri-O-methylglucose. Characterisation was achieved by conversion into the aniline derivative which, after recrystallisation from ether-light petroleum, had m.p. and mixed m.p. = 145-6° (with an authentic specimen of 2,3,4-tri-O-methyl-N-phenyl-D-glucosylamine, m.p. = 149-50°).

Partial hydrolysis of gum ghatti.

Preparation of the gum acid.

Crude gum ghatti (50 g.) in water (1 l.) was dissolved as completely as possible by stirring for two days. Insoluble materials were removed by centrifugation followed by filtration of the centrifugate through glass fibre paper. Concentrated hydrochloric acid (10 ml.) was added to the filtrate and the solution was poured slowly with stirring into ethanol (4 l.). The precipitated polysaccharide was dissolved in water (1 l.) containing concentrated hydrochloric acid (20 ml.) and the solution again poured into ethanol (4 l.). The re-precipitated polysaccharide was dissolved in water (1 l.) and chloride ions removed by dialysis against running tap water for five days. The solution was concentrated (500 ml.) and the purified gum acid precipitated by slowly pouring into acetone (3 l.). The polysaccharide was removed at the centrifuge and was dried by solvent exchange in acetone (5 x 500 ml.) and finally in ether (5 x 300 ml.). Excess organic solvent was removed by desiccation in vacuo and after four days the polysaccharide was powdered (42 g.).

Trial experiment

Trial hydrolyses of the gum acid were undertaken to determine the optimum conditions of hydrolysis to give a maximum yield of the tetrasaccharide

$$\underline{D}\text{-}\underline{Gp}A\ 1\rightarrow 2\ \underline{D}\text{-}\underline{Man}p\ 1\rightarrow 4\ \underline{D}\text{-}\underline{Gp}A\ 1\rightarrow 2\ \underline{D}\text{-}\underline{Man}$$

Gum acid (0.250 g.) was dissolved in water (13 ml.) and 2 N-sulphuric acid (13 ml.) was added. The solution was heated on a boiling-water bath for eight hours. Samples (1 ml.) were removed every thirty minutes and high molecular weight material, if any, precipitated in ethanol (4 ml.). After centrifugation each supernatant solution was neutralised and concentrated to give a syrup which was examined chromatographically in solvents A, B and C. The results of this experiment showed that the optimum conditions of hydrolysis for the formation of the above tetrasaccharide involved N-sulphuric acid at 100° for four and a half hours.

Large scale hydrolysis.

Gum acid (40 g.) was dissolved in boiling water (3 l.) and hot 4 N-sulphuric acid (1 l.) was added. The solution was heated on a boiling-water bath for four and a half hours. The hydrolysate was allowed to cool and was neutralised with saturated barium hydroxide and solid barium carbonate. Insoluble material was removed at the centrifuge and washed with water (4 x 250 ml.). The supernatant solution and water washings were combined and concentrated (400 ml.). After removal of cations the hydrolysate was further concentrated to a syrup (32 g.).

Chromatographic examination of the hydrolysate in solvents A, B and C indicated large amounts of galactose, arabinose and xylose, with lesser amounts of mannose, glucuronic acid and a number of oligosaccharides, the majority of which were acidic.

Fractionation of the hydrolysate.

A DEAE-sephadex column (27 x 3 cm., 40 g.) in the formate form was prepared in the normal way. The hydrolysate, in water (50 ml.), was applied to the column in four batches, each batch being washed in with an equal quantity of water, before the next was added. After allowing the sugars to soak into the column overnight, the neutral components were eluted with water (ca. 3 l.), elution being continued until the eluate no longer gave a positive reaction towards the phenol-sulphuric acid reagent. Concentration of the eluate gave a dried syrup (26.5 g.) which on examination by paper chromatography in solvents A, B and C was shown to contain galactose and arabinose, with smaller quantities of xylose and mannose.

The acidic sugars were fractionated as follows. The column was first eluted with 0.05 M formic acid solution, a bulk fraction being collected. Fractions (20 ml.) were then collected every thirty minutes by gradient elution with water containing formic acid (0.05 M $\longrightarrow$ 0.5 M). The elution pattern was followed by concentration of the contents of every third tube and chromatographic examination in solvents B and C. The similar fractions were bulked, concentrated (40 ml.) and extracted with ether in a liquid-liquid extractor for eighteen hours. The formic acid free solutions were filtered and further concentrated to dry syrups. The column was finally eluted with formic acid solution (10% in water); this, however, failed to yield any further oligosaccharides. By the above procedure seven fractions were collected in all, and the

elution sequence as a whole may be represented as below (Table XIII).

Table XIII

Eluant (formic acid solution)	Tubes	Fraction	Contents	Weight (in g.)
0.05 M	Bulk (2.5 l.) 1 - 69	A	glucuronic acid, oligosaccharides I and II	3.40
0.05 → 0.1 M	70 - 125			
	126 - 217	B	no sugars detected	-
	218 - 295			
0.1 → 0.5 M	296 - 350	C	oligosaccharides I, II and IV	0.20
	351 - 420	D	oligosaccharides I, II, III and IV	0.13
0.5 M	421 - 550	E	oligosaccharides I and IV	0.107
	551 - 650	F	oligosaccharide I	0.07
10%	Bulk (1 l.)	G	no sugars detected	-

Oligosaccharides I and II had R_{gal} values of 0.40 and 0.23 respectively in solvent B. Oligosaccharides III and IV had R_{gal} values of 0.48 and 0.34 respectively in solvent C.

Fraction A

This fraction (3.4 g.) on chromatographic examination in solvents B and C appeared to consist of three sugars one of which was glucuronic acid and the other two had the chromatographic mobilities of oligosaccharides I and II. A small sample of the fraction (0.2 g.) was fractionated into the individual components by filter sheet chromatography in solvent B, thus giving chromatographically pure oligosaccharides I and II and glucuronic acid.

Fraction C

This fraction contained three sugars whose chromatographic mobilities were identical to those of oligosaccharides I, II and IV. The fraction was separated into its components by filter sheet chromatography in solvent C.

Fraction D

This fraction was similar to fraction C, except that a further component oligosaccharide III was now present. Oligosaccharides III and IV were isolated from this fraction by filter sheet chromatography in solvent C.

Fraction E

Filter sheet chromatographic separation of this fraction in solvent B gave oligosaccharides I and IV.

Fraction F

This fraction contained chromatographically pure oligosaccharide I as its only component.

Oligosaccharide I

This sugar obtained from fraction F (0.07 g.) was chromatographically indistinguishable from 2-O-(β-D-glucopyranosyluronic acid)-D-mannose and had R_{gal} = 0.40 and 0.76 in solvents B and C respectively. Hydrolysis of a sample (2 mg.) gave glucuronic acid and mannose in approximately equal amounts (visual examination of paper chromatogram).

A small sample (5 mg.) was methylated by the Kuhn procedure. The product was methanolysed and after gas-liquid chromatographic examination of the methyl glycosides in system (C), the following sugars were shown to be present: 2,3,4-tri-O-methylglucuronic acid (T = 3.16, 2.40) and 3,4,6-tri-O-methylmannose (T = 2.78).

Oligosaccharide II

This sugar (0.04 g.) isolated from fraction A (0.120 g.) was chromatographically indistinguishable from 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, and had R_{gal} = 0.20 and 0.51 in solvents B and C respectively. Hydrolysis gave glucuronic acid and galactose. Further investigation was not carried out.

Oligosaccharide III

This sugar (0.020 g.) obtained from fraction E (0.107 g.) had R_{gal} = 0.48. Hydrolysis gave galactose and glucuronic acid. Further investigation was not carried out.

Oligosaccharide IV

This sugar (0.08 g.) isolated from fractions D and E was chromatographically indistinguishable from O-(β-D-glucopyranosyluronic acid)-(1→2)-O-α-D-mannopyranosyl-(1→4)-O-(β-D-

glucopyranosyluronic acid)-(1 → 2)-D-mannose, isolated from leiocarpan A, having R_{gal} = 0.34 (solvent D).

A small sample (2 mg.) was hydrolysed with N-sulphuric acid (1 ml.) at 100° for five hours. Examination of the hydrolysate in solvent B showed the presence of mannose, glucuronic acid and 2-O-(β-D-glucopyranosyluronic acid)-D-mannose.

Reduction of the derived methyl ester methyl glycosides with sodium borohydride and hydrolysis gave glucose and mannose in approximately equal proportions (visual examination of paper chromatogram).

A sample of the sugar (5 mg.) was reduced with sodium borohydride and the derived glycitol hydrolysed by 0.5 N-sulphuric acid at 100° for one hour. Chromatographic examination of the hydrolysate in solvent I (one week) showed the presence of both 2-O-(β-D-glucopyranosyluronic acid)-D-mannose and 2-O-(β-D-glucopyranosyluronic acid)-D-mannitol.

Measurement of the sugar content, by the phenol-sulphuric acid method, both before and after reduction showed that one sugar residue in four was removed as a result of reduction, thus indicating that this oligosaccharide was in fact a tetrasaccharide.

The oligosaccharide (0.02 g.) was dissolved in water (1 ml.) and treated with methyl sulphate (1 ml.) and 30% (w/v) aqueous sodium hydroxide (2 ml.) in an atmosphere of nitrogen, the reagents being added dropwise, with stirring, over a period of four hours. Further similar additions of reagents were

made on five successive days after which the final reaction mixture was heated on a boiling-water bath for one hour to destroy the unreacted methyl sulphate. After allowing to cool, the reaction mixture was acidified (pH 4) with 2 N-sulphuric acid, and most of the sodium sulphate precipitated by pouring the aqueous solution (22 ml.) into ethanol (500 ml.). The insoluble inorganic salts were removed at the centrifuge and the centrifugate was concentrated to a syrup which was dissolved in water (10 ml.) and extracted with chloroform (5 x 25 ml.). The dried chloroform extracts were combined and concentrated to give a syrup (0.012 g.). This product was dissolved in methyl iodide (1 ml.) and silver oxide (0.01 g.) was added in portions over four hours, the reaction mixture being stirred under reflux both during, and for eighteen hours after the addition of the silver oxide. The reaction mixture was then filtered, and the residue extracted with boiling chloroform in a Soxhlet extractor. The filtrate and chloroform extracts were combined and concentrated to a syrup. Two such Purdie methylations were carried out after which a small sample (2 mg.) of the methylated product (0.009 g.; OMe 48.2%) was methanolysed and the methyl glycosides examined by gas-liquid chromatography. This gave evidence for the presence of the following sugars: 2,3,4-tri- (τ = 3.19 and 2.46) and 2,3-di-O-methyl-D-glucuronic acid (τ = 7.30, 8.22 and 10.05), and 3,4,6-tri-O-methyl-D-mannose (τ = 2.83).

The remainder of the methylated oligosaccharide (0.01 g.) was dissolved in tetrahydrofuran (2 ml.) and lithium aluminium

hydride (0.01 g.) in tetrahydrofuran (2 ml.) was added. The reaction mixture was stirred for one hour at room temperature and then under reflux for a further three hours. Excess lithium aluminium hydride was destroyed by the addition of ethyl acetate saturated with water, and after the mixture had been acidified (pH 4) by the addition of 2 N-sulphuric acid it was extracted with chloroform (4 x 25 ml.). The dried chloroform extracts were combined and concentrated to a syrup (0.006 g.). Found: OMe 44.8%.

The remainder of the reduced methylated product (3 mg.) was methanolysed and after gas-liquid chromatography of the methyl glycosides in system (C), the presence of the following sugars was indicated: 2,3,4-tri- (τ = 2.30, 3.26) and 2,3-di-O-methyl-D-glucose (τ = 9.2, 12.7) and 3,4,6-tri-O-methyl-D-mannose (τ = 2.74).

Leiocarpan B

Methylation analysis

A sample of leiocarpan B (1.9 g.) was methylated by six additions of methyl sulphate (20 ml.) and 30% (w/v) aqueous sodium hydroxide (40 ml.) in an atmosphere of nitrogen. After the last addition the reaction mixture was heated on a boiling-water bath to destroy any unreacted methyl sulphate. It was then acidified (pH 4) by the addition of 2 N-sulphuric acid and the partly methylated product extracted into chloroform (5 x 250 ml.). The dried chloroform extracts were combined, concentrated (20 ml.) and poured into light petroleum (500 ml.). The precipitated, partly methylated polysaccharide was isolated at the centrifuge and dried in vacuo (1.38 g.). Found: OMe = 37%.

This partly methylated acidic polysaccharide was dissolved in water (100 ml.) and treated with silver carbonate (0.1 g.). After removal of insoluble material by filtration through glass fibre paper, the filtrate was freeze-dried to give the silver salt (1.4 g.), which was dissolved in iodomethane (20 ml.), and silver oxide (0.14 g.) was added over four hours. The mixture was stirred under reflux both during, and for eighteen hours after, the addition of silver oxide. The reaction mixture was then filtered and the residue was extracted with boiling chloroform in a Soxhlet extractor. The filtrate and chloroform extracts were combined and concentrated to a syrup. Three further Purdie methylations were carried out, the last of which

failed to raise the methoxyl content of the methylated product (0.7 g.). Found: OMe = 42.1%; $[\alpha]_D = -46.5^\circ$ (c = 1.07 in $CHCl_3$).

Reduction of methylated leiocarpan B

Methylated leiocarpan B (0.41 g.) was dissolved in tetrahydrofuran (20 ml.) and lithium aluminium hydride (0.41 g.) in tetrahydrofuran (20 ml.) was added. The reaction mixture was stirred at room temperature for thirty minutes and then under reflux for three hours. Excess lithium aluminium hydride was destroyed by the addition of ethyl acetate saturated with water, and after acidification (pH 4) by the addition of 2 N-sulphuric acid, the reduced methylated polysaccharide was extracted into chloroform (5 x 250 ml.). The dried chloroform extracts were combined, concentrated (20 ml.) and poured into light petroleum (500 ml.). The precipitate was removed at the centrifuge and dried in vacuo to give the reduced methylated leiocarpan B (0.320 g.). Found: OMe = 40.5%, $[\alpha]_D = -37.5^\circ$ (c = 1.5 in $CHCl_3$).

Samples (20 mg.) both of the methylated and of the reduced methylated leiocarpan B were methanolysed and the products examined by gas-liquid chromatography. The results of this are shown below (Table XIV).

Table XIV

Methyl glycoside of:	Relative amount	T values		
		Column a	Column c	Column d
2,3,5-Me_3 arabinose	++++	(0.56),0.71	(0.47),0.63	(0.61),0.78
2,5-Me_2 arabinose	++	1.84, 3.39	1.49,(2.76)	2.20
2,4-Me_2 arabinose	+	2.30	2.00	
2,3-Me_2 arabinose	+	1.52	1.19	(1.86)
2,3,4-Me_3 xylose	+++	0.45,(0.56)	0.41,(0.48)	0.47,(0.61)
2,3,4,6-Me_4 galactose	+		1.79	(1.85)
2,3,4-Me_3 galactose	++	(7.25)	6.78	(8.7)
2,4,6-Me_3 galactose	++	4.07, 4.63	3.60, 4.15	4.69, 5.33
2,4-Me_2 galactose	++			
2,3-Me_2 glucuronic acid[/]	+++		7.76, 9.55	
2,3,4-Me_3 glucose[*]	++		3.38	
2,3-Me_2 glucose[*]	+++		9.17,12.2	
3,4-Me_2 mannose	+++	(7.3)	5.77	(8.7)

[/] Present as methyl esters.

[*] Present after reduction of the methylated polysaccharides.

In those cases where the relative retention time may be attributed to more than one sugar, the T value is given in parentheses.

Evidence was also obtained for the presence of the following sugars,

2,3,4-tri-O-methylarabinose,

3,5-di-O-methylarabinose,

2,3,4-tri-O-methylglucuronic acid,*

and 3,4-di-O-methylmannose.

Due to the overlapping of peaks, however, definite conclusions as to which, if not all, of these are present, cannot be ascertained.

In addition the following sugars were detected by paper chromatography in solvent F,

4-O-methylmannose,

3-O-methylglucose,

2-O-methylgalactose,

2-O-methylarabinose,

2,6-di-O-methylgalactose.

Partial acid hydrolysis

Leiocarpan B (0.5 g.) was dissolved in water (25 ml.) and the solution heated on a boiling-water bath at 100°. To the hot solution was added 2 N-sulphuric acid (25 ml.) and the resulting solution was heated at 100° for five hours. After allowing to cool the hydrolysate was poured into ethanol (200 ml.). A very small amount of polysaccharide was precipitated. This was removed at the centrifuge, washed with a solution of ethanol in water (80%), and discarded. The centrifugate and washings were combined, concentrated (25 ml.) and neutralised by the addition of saturated barium hydroxide and solid barium carbonate. The precipitated barium salts

were removed at the centrifuge and washed with water (3 x 50 ml.). The centrifugate and washings were combined and after removal of cations were concentrated to a syrup (0.420 g.).

A preliminary chromatographic examination of the hydrolysate in solvents A, B and C indicated the presence of the following monosaccharides in the relative amounts shown in parentheses: D-galactose (+++), L-arabinose (++), D-xylose (++), D-mannose (trace), L-rhamnose (trace) and D-glucuronic acid (trace). In addition to these monosaccharides a number of oligosaccharides were also present.

The hydrolysate was placed on a DEAE-sephadex column (20 x 1 cm.; 5 g.) in the formate form, prepared in the normal way. The column was allowed to stand overnight and then the neutral sugars were eluted with water until the eluate gave a negative reaction to the phenol-sulphuric acid reagent (ca. 500 ml. water). This neutral fraction was concentrated to a syrup (0.306 g.) which on examination by paper chromatography in solvents A, B and D showed the presence of the monosaccharides D-galactose, D-mannose, L-arabinose, D-xylose and L-rhamnose in the same relative proportions as shown above.

The acidic sugars then remaining on the column were eluted by 3% formic acid solution, again until the eluate gave a negative reaction to the phenol-sulphuric acid reagent (ca. 200 ml.). After concentration (20 ml.) this fraction was extracted with ether for eighteen hours in a Soxhlet extractor, to remove formic acid. The formic acid free solution was filtered and further concentrated to a syrup (0.073 g.).

Chromatographic examination of this syrup in solvents B and C, against authentic standard sugars, showed the following components in the relative amounts as shown.

Component sugar	Amount	R_{GpA} (solvent B)
D-GpA	+	1.00
D-GpA 1 → 2 D-Man	++	0.37
D-GpA 1 → 6 D-Gal	+++	0.16
D-GpA 1 → 2 D-Manp 1 → 4 D-GpA 1 → 2 D-Man	+	0.04
Unknown	trace	0.09

Autohydrolysis

Leiocarpan B (0.200 g.) was dissolved in water (20 ml.) and the solution was heated at 90° for five days. Samples of the autohydrolysate (1 ml.) were removed at various intervals of time according to the formula (0.5×2^n) hours. These samples were poured into ethanol (5 ml.) and the precipitated degraded gum was removed at the centrifuge. Each centrifugate was concentrated to a syrup which was examined by paper chromatography in solvents A, B and D.

In addition to those neutral oligosaccharides present after the partial acid hydrolysis, the following neutral oligosaccharides were shown to be present by a comparison of their chromatographic mobility with that of authentic standard sugars.

	R_{gal}
3-O-β-L-arabinopyranosyl-L-arabinose	0.85
3-O-β-L-arabinofuranosyl-L-arabinose	1.40
3-O-β-D-galactopyranosyl-L-arabinose	0.65
3-O-β-D-galactopyranosyl-D-galactose	0.49
6-O-β-D-galactopyranosyl-D-galactose	0.30

As a control, exactly parallel experiments were carried out on leiocarpan A and unfractionated A. leiocarpus gum acid. The results of these experiments showed that while the same series of oligosaccharides were produced in all three cases, in leiocarpan A very small amounts of the two galactobioses were obtained.

PART II

ACACIA MEARNSII GUM

INTRODUCTION AND DISCUSSION

INTRODUCTION

In some cases it has been observed[7] that there are slight variations in composition and physical constants, between samples of the same gum but of different origin; that is to say from different trees, situated either in the same or in perhaps widely separated geographical locations. Since a sample of Acacia mollissima gum of South African origin had previously been investigated by Stephen[84,85], it was decided to carry out investigations on a sample of the same gum but of different origin. The gum, of West Indian origin, which was received as Acacia decurrens var. mollis (syn. A. mollissima) gum, had been collected from a single tree through the kindness of Dr. Jean Tyler. Since receiving the gum, the name of the species from which both samples of gum were obtained has been changed to Acacia mearnsii and it is under this name that investigations will now be reported.

Preliminary investigations[50] showed that the gum on autohydrolysis gave D-galactose, L-arabinose and L-rhamnose, and that in addition to these monosaccharides, the following disaccharides were liberated:

3-O-β-D-galactopyranosyl-D-galactose,

6-O-β-D-galactopyranosyl-D-galactose,

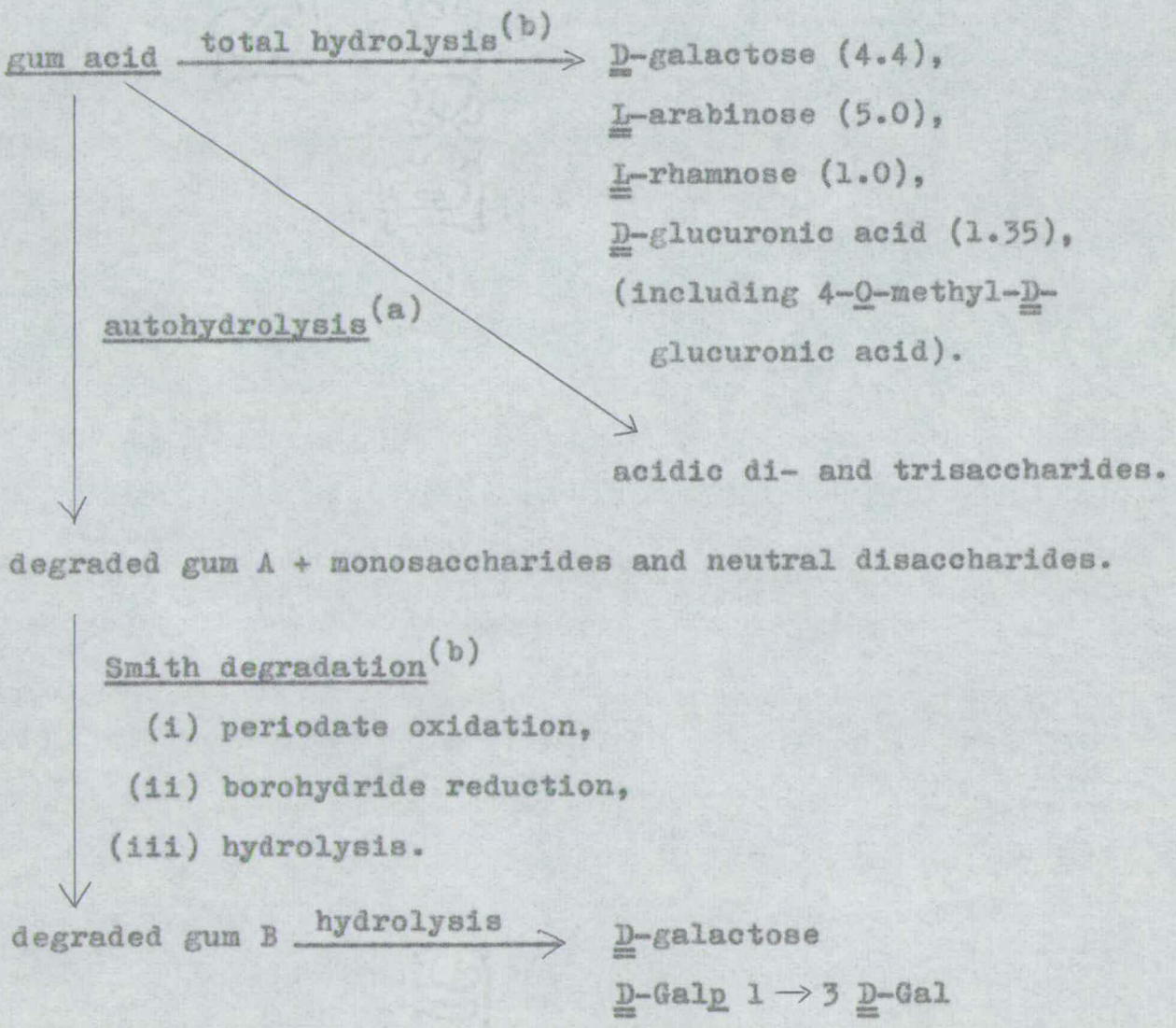
3-O-β-L-arabinopyranosyl-L-arabinose.

These disaccharides were characterised as crystalline sugars or their derivatives. The resulting degraded gum, known as degraded gum A, was found to be arabinose free and as

a result of methylation analysis was shown to have a highly branched character. Furthermore methylation studies of the whole gum gave information about the substitution pattern in the undegraded gum.

The pathways by which structural studies on A. mearnsii gum have been carried out, apart from methylation analyses, both during the preliminary investigation (a), and also during a recent additional examination (b), may be schematically represented as below (Fig. VIII).

Figure VIII



DISCUSSION

Purification and assessment of homogeneity

The sample of gum was the same as was used for previous investigations[50]. While the bulk of the gum was soluble in cold water, a small amount remained and was dissolved, either in hot water or in dilute ammonia. The solutions of gum obtained by this treatment were filtered, precipitated twice in ethanol, dissolved in water and freeze-dried. The polysaccharide which was obtained by dissolution of the gum in cold water had $[\alpha]_D = -61^0$, uronic acid anhydride content of 12.1% (by decarboxylation). Only this fraction was used in subsequent investigations.

Examination of the purified gum acid by chromatography on DEAE-cellulose in the phosphate form showed that it was homogeneous. One fraction was obtained and this (uronic acid anhydride content = 12.7%, by carbazole determination) accounted for approximately 80% of all polysaccharide originally applied to the column. A second very small fraction (approx. 3% of original polysaccharide) which was eluted by the weakest buffer was found to have negligible uronic acid content (by carbazole) and showed the presence of monosaccharides on paper chromatographic examination.

Composition of gum acid

A mild hydrolysis designed so as to preferentially remove peripheral residues gave arabinose and rhamnose along with a

trace of galactose. Separation of the sugars by thick paper chromatography and determination of their amounts by the phenol sulphuric acid method showed that the arabinose to rhamnose ratio was 5:1. The degraded gum obtained by this process was free of arabinose and contained only residues of galactose and glucuronic acid (or its 4-methyl ether).

Under much stronger hydrolysis conditions, designed to break up the molecule to at least as far as aldobiouronic acids, the gum gave galactose, arabinose, rhamnose and a number of acidic components. The hydrolysate was again separated by thick paper chromatography and the amounts of the sugars estimated colorimetrically as above. Correction of the estimated amounts a) due to degradation of rhamnose on prolonged hydrolysis and b) due to incomplete breakdown of aldobiouronic acids, gave the D-galactose : L-arabinose : L-rhamnose : D-glucuronic acid ratio as 4.4:5:1:1.35.

Graded hydrolysis

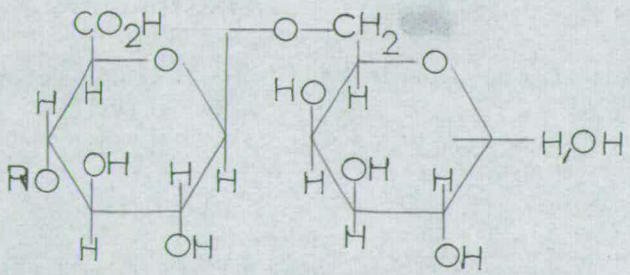
Partial hydrolysis of the gum by N-sulphuric acid at 90-95° for five hours gave a mixture of mono- and oligo-saccharides. Column chromatography of the hydrolysate on anion exchange Sephadex was used to separate the acid and neutral components. Paper chromatographic separation of the acidic sugars gave two monosaccharides and four oligo-saccharides.

One of these oligosaccharides was chromatographically indistinguishable from 6-O-(β-D-glucopyranosyluronic acid)-

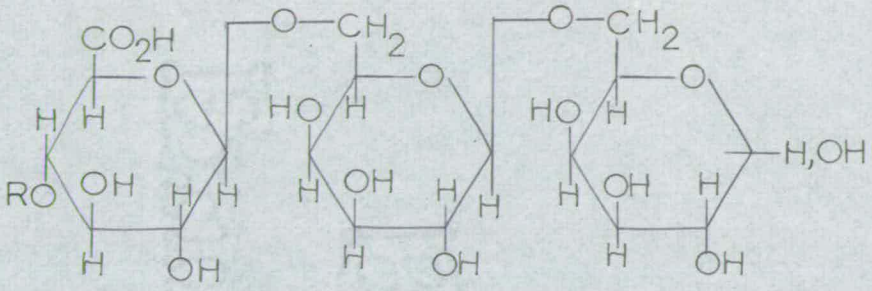
D-galactose. Hydrolysis gave glucuronic acid and galactose. Reduction of the derived methyl ester methyl glycosides with sodium borohydride followed by hydrolysis gave glucose and galactose. The oligosaccharide was methylated and examination of the methanolysis products from the methylated derivative by gas-liquid chromatography showed the presence of the methyl glycosides of 2,3,4-tri-O-methyl-glucuronic acid, and 2,3,4- and 2,3,5-tri-O-methyl galactose.

By an analogous series of experiments a second component was shown to be the 4-methyl ether of the above, namely 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. The specific rotations of the above two oligosaccharides, being -7.5° and $+1^{\circ}$ respectively, were consistent with the presence of β-linkages.

The two additional acidic oligosaccharides were characterised as aldotriouronic acids. One of these by partial hydrolysis and methylation studies was characterised as O-(β-D-glucopyranosyluronic acid)-(1→6)-O-β-D-galactosyl-(1→6)-D-galactose, and the other was tentatively characterised as the 4-O-methyl-D-glucuronic acid analogue. The four acidic oligosaccharides are therefore as depicted below (a and b).



(a)



(b)

R = - H or - CH_3

On autohydrolysis the gum liberated, in addition to monosaccharides, three neutral oligosaccharides which by the crystallinity of the sugars or of some derivative were characterised as

3-O-β-D-galactopyranosyl-D-galactose

6-O-β-D-galactopyranosyl-D-galactose

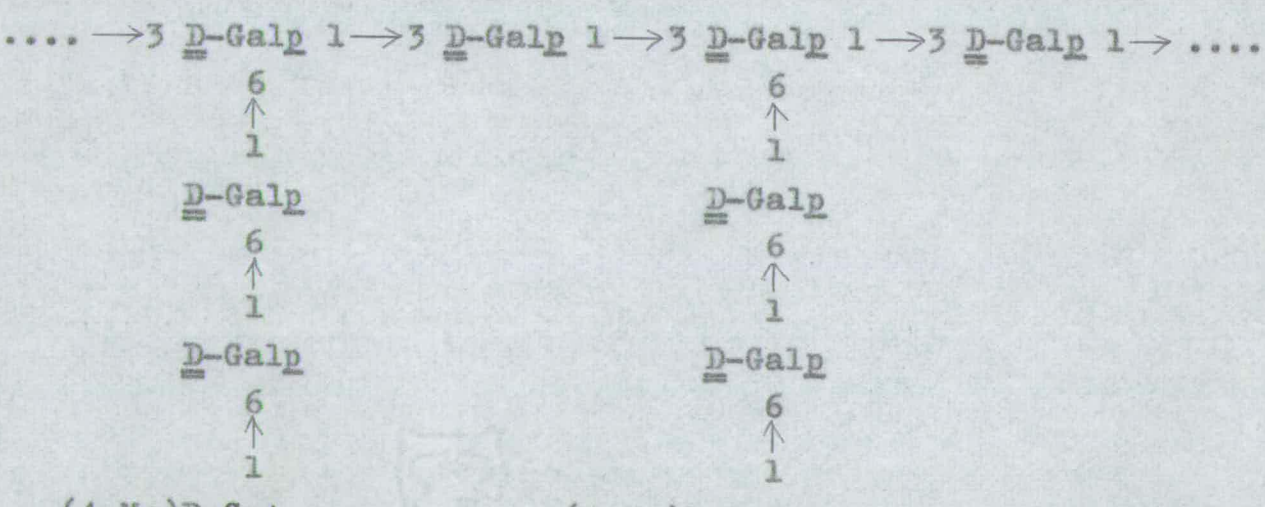
3-O-β-L-arabinopyranosyl-L-arabinose

The degraded A. mearnsii gum (uronic acid anhydride content = 19.5% - by decarboxylation) obtained from the autohydrolysis mixture was converted to its fully methylated derivative, which on hydrolysis gave 2,3,4,6-tetra-, 2,3,4- and 2,4,6-tri-, and 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid. On the assumption that the methylation is complete, the locations of the hydroxyl groups in the five cleavage products therefore indicate the way in which the monosaccharide units are linked together. Thus the isolation of 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid indicates that D-galactose and D-glucuronic acid are present as end groups. Similarly by the

isolation of 2,3,4- and 2,4,6-tri-O-methyl-D-galactose we have indications of both 1,3- and 1,6-linked galactose residues. Furthermore the isolation of 2,4-di-O-methyl-D-galactose indicates the nature of the galactose unit which occurs at the branch point.

The methylation analysis therefore gives an indication of the branched nature of the degraded gum A. The five cleavage products isolated mean that not only are all the residues in the pryanose form, but, as well as this, they are all linked to one another via 1→3 or 1→6 linkages. Addition evidence concerning this galactan framework comes from partial hydrolysis experiments from which both 1→3 and 1→6 linked galactobioses have been isolated.

From the results of methylation and partial hydrolysis studies many partial structures may be advanced for degraded gum A. The number of these is however limited by the isolation of the two aldotriouronic acids and while the following (A) is only one of the many partial structures which are possible, it does illustrate the known structural features of degraded gum A.



(A)

Smith degradation studies

Degradation of the degraded gum A by Smith's procedure[21] gave degraded gum B which was examined both by partial hydrolysis and also by methylation analysis.

Partial hydrolysis of degraded gum B followed by examination of the hydrolysate by paper chromatography in comparison with authentic standard oligosaccharides, indicated the presence of

3-O-β-D-galactopyranosyl-D-galactose

O-β-D-galactosyl-(1→3)-O-β-D-galactosyl-(1→3)-D-galactose and higher oligosaccharides. The disaccharide 6-O-β-D-galactopyranosyl-D-galactose was noticeably absent as also was its homologous trisaccharide.

Methylation of degraded gum B was found to proceed with difficulty and had to be carried out in a number of stages. It was finally methylated to such a degree, however, that further treatment with Purdie's reagents failed to raise the methoxyl content. Despite possible incomplete methylation examination of the cleavage products from hydrolysis, by paper chromatography of the sugars and by gas-liquid chromatography of the methyl glycosides indicated the presence of 2,3,4,6-tetra-, 2,4,6-tri- and 2,6-di-O-methyl-D-galactose. Of these sugars the 2,4,6-tri-O-methyl galactose was by far the most abundant and the trace of dimethyl galactose is probably a product of undermethylation, since other workers[36] have reported difficulty in methylating polysaccharides which approximated to a 1→3 linked galactan.

The results of the Smith degradation of the degraded gum A therefore suggest that the main chain of the molecule is composed mainly if not exclusively of 1→3 linked D-galactopyranose residues to which other D-galactopyranose residues linked 1→6 are attached as side chains. These results are therefore in support of the above partial structure (A).

Methylation analysis

The hydrolysis of methylated A. mearnsii gum gave rise to a large number of methylated monosaccharides. In addition to those which had already been obtained from the degraded gum A, the following were shown to be present in the whole gum: 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5- and 2,3,4-tri-, 2,3- and 2,5-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-D-glucuronic acid. The isolation of these products showed that the labile residues L-rhamnose, L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose were joined to the stable nucleus in the form of L-arabinofuranose or L-rhamnopyranose.

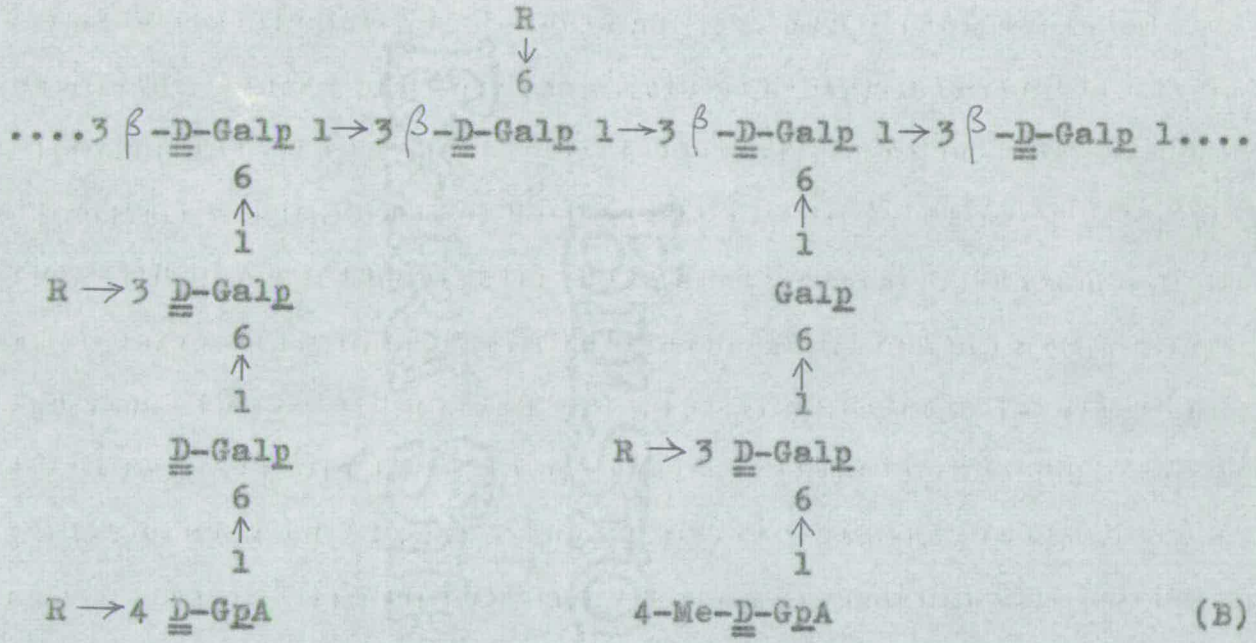
On consideration of the amount of 2,3,4-tri-O-methyl-D-galactose as compared with that of 2,4-di-O-methyl-D-galactose in the methylated gum and the methylated degraded gum A, it would appear that some at least of these labile residues are attached to the stable framework via C_3 of the 1→6 linked galactose residues in the side chains. Also the D-glucuronic acid residues afford both 2,3,4-tri- and 2,3-di-O-methyl-D-glucuronic acid and so some of the labile residues would thus appear to be attached to C_4 of the glucuronic acid. (In the

light of this it is interesting to note the isolation of 4-O-α-L-rhamnopyranosyl-D-glucose from the acetolysis of carboxyl reduced gum arabic[19].) The tri-O-methyl-D-glucuronic acid probably arises from end group 4-O-methyl-D-glucuronic acid.

Additional information on the positions of attachment of the labile residues is obtained on consideration of the increase in the proportion of 2,4,6-tri-O-methyl-D-galactose in the methylated degraded gum, and the corresponding decrease in the proportion of 2,4-di-O-methyl-D-galactose. From this it seems that a large proportion of the acid labile groups must be attached to position 6 of those galactose residues in the main chain which do not carry a $1 \rightarrow 6$ linked galactose side chain. Further evidence on this point lies in the increase in the proportion of 2,3,4,6-tetra-O-methyl-D-galactose accompanied by a decrease in the proportion of 2,3,4-tri-O-methyl-D-galactose, on comparing the methylated and methylated degraded polysaccharides. Further acid-labile groups are thus attached to position 6 of D-galactopyranose residues present as side chains in the gum.

In summary therefore, assuming partial structure A to be an accurate representation of degraded gum A, it appears there are three positions to which labile residues may be attached in the whole gum. These are, at C_4 of the glucuronic acid residues which are not already methyl etherified, at C_3 of the galactose residues in the $1 \rightarrow 6$ linked side chains, and at C_6 of the main chain galactose residues which do not already carry

side chains (see partial structure B below).



(B)

where R = L-Araf 1.... , L-Rhap 1.... ,
or occasionally β-L-Arap 1→3 L-Araf 1-....

While the above, (B), represents the known structural features of A. mearnsii gum, since only some of the galactose residues carry substituents, and since it is as yet impossible to say which of these in fact are preferentially substituted, partial structure B represents only one of the structures which may be advanced.

Comparison of West Indian sample with that of South African origin

Since detailed structural investigations of the type outlined above have as yet not been reported on a sample of South African origin, a direct structural comparison is not possible. Nevertheless a number of physical constants are available for

two South African samples[84,85] and these may be compared with the known physical constants for the sample of West Indian origin (Table VII).

Table VII

Sample of *A. mearnsii* gum	$[\alpha]_D$	Equivalent (titration)	Equivalent (uronic acid content)	Methylated derivative	
				$[\alpha]_D$	O Me
South African sample I[84]	-49°	1880	-	-	-
South African sample II[85]	-55°	1920	1730	-66°	42.6%
West Indian sample	-61°	-	1450	-66°	42.4%

Comparison may also be made on the basis of composition of the gums and of their methylated derivatives (Tables VIII and IX).

Table VIII

Sample of *A. mearnsii* gum	D-galactose	L-arabinose	L-rhamnose	D-glucuronic acid (inc. its 4-methyl ether)
South African sample I	5	6	1	1
West Indian sample	4.4	5	1	1.35

Furthermore both the West Indian sample and the South African

sample I[86] give rise to 3-O-β-L-arabinopyranosyl-L-arabinose. Although the isolation of 4-O-methyl-D-glucuronic acid was not reported, a value of 0.35% was obtained for the methoxyl content of the South African sample I, and so it seems probable that this gum also contains 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Table IX

Sugar	Proportions of sugars	
	South African sample II	West Indian sample
2,3,4-tri-O-methyl-rhamnose	+	+
2,3,5-tri-O-methyl-arabinose	++++	++++
2,3,4-tri-O-methyl-arabinose	+	+
2,3-di-O-methyl-arabinose	+++	+++
2,5-di-O-methyl-arabinose	++++	++++
2,3,4,6-tetra-O-methyl-galactose	++	trace
2,4,6-tri-O-methyl-galactose	++	++
2,3,4-tri-O-methyl-galactose	+++	+
2,4-di-O-methyl-galactose	++++	++++
2,3,4-tri-O-methyl glururonic acid	++	++
2,3-di-O-methyl glucuronic acid	++	++

The relative proportions of the above sugars were estimated visually, after gas-liquid chromatographic examination of the methanolysis products of the methylated polysaccharides. As is seen from the table all the methylated sugars present in one gum

were present in the other, but differences were observed in the relative amounts of some of these components, thus suggesting that the actual amount of substitution, that is the number of side chains, may be different.

Comparison of the structural features of *A. mearnsii* gum with those of *A. senegal* and *A. pycnantha* gums

A. mearnsii gum shows distinct similarity to both gum arabic (*A. senegal*) and to *A. pycnantha* gum on account of the fact that in all three gums, the autohydrolysis degraded gum has a main chain of D-galactopyranose residues joined mainly if not exclusively by 1→3 linkages, and to which further D-galactopyranose residues linked 1→6, are attached as side chains. These side chains, in the degraded gums being terminated by uronic acid residues.

The three gums show further similarity in the release of L-arabinofuranosyl and L-rhamnopyranosyl residues on mild hydrolysis, but by the same process they differ, in the nature of the more complex oligosaccharides which are liberated from the peripheral parts of the molecule (see Table X).

It has been established that some if not all of the L-rhamnopyranose residues in gum arabic are attached to position 4 of D-glucuronic acid[19]. Similar evidence has not yet been obtained for either *A. mearnsii* or *A. pycnantha* gums but on the basis of present evidence the possibility exists that this is in fact the case. The situation in *A. mearnsii* gum is, however, complicated by the presence of 4-O-methyl-D-

glucuronic acid, but after allowance has been made for this, enough D-glucuronic acid remains to accommodate some if not all of the rhamnose. As with *A. pycnantha* gum it is only on the application of acetolysis to the carboxyl reduced polysaccharides that this question will be resolved.

Despite obvious differences in the nature of some of the labile peripheral units, it is already clear that these three gums show distinct similarity to one another, and from early results on other *Acacia* gums[7] it seems probable that this similarity extends throughout the genus.

Table X

Species	Molar percentages of monosaccharides				Peripheral fragments	Acidic fragments
	D-Gal	L-Ara	L-Rha	D-GpA		
A. senegal	38	38	12	12	Araf 1-... Rhap 1-... Arap 1 $\overset{\beta}{\rightarrow}$ 3 Araf 1-... Galp 1 $\overset{\alpha}{\rightarrow}$ 3 Araf 1-...	Rhap 1 $\overset{\alpha}{\rightarrow}$ 4 GpA 1 $\overset{\beta}{\rightarrow}$ 6 Gal
A. pycnantha	67	25	2	5	Araf 1-... Rhap 1-... Araf 1 $\overset{\beta}{\rightarrow}$ 3 Araf 1-...	GpA 1 $\overset{\beta}{\rightarrow}$ 6 Gal
A. mearnsii	37	43	8	12	Araf 1-... Rhap 1-... Arap 1 $\overset{\beta}{\rightarrow}$ 3 Araf 1-...	...4 GpA 1 $\overset{\beta}{\rightarrow}$ 6 Gal 4-O-Me-GpA 1 $\overset{\beta}{\rightarrow}$ 6 Gal

EXPERIMENTAL

EXPERIMENTAL

Isolation and purification of *Acacia mearnsii* gum

The crude gum was obtained as light brown, hard glassy nodules, to which pieces of bark and other extraneous materials were attached. This crude gum (18 g.) was crushed to a powder, and allowed to swell in water (3 l.) overnight. This gummy suspension was stirred for two days after which it was centrifuged. The centrifugate was filtered, concentrated to small volume (250 ml.), acidified by the addition of concentrated hydrochloric acid (2.5 ml.), and poured slowly, with stirring, into ethanol (1 l.). The precipitated polysaccharide, after reprecipitation, was dissolved in water (1 l.) and the solution was dialysed against tap water for four days. The non-diffusate was concentrated to small volume (100 ml.) and freeze-dried to give polysaccharide I (13.7 g.), $[\alpha]_D = -61^\circ$ ($c = 0.90$); uronic acid anhydride content = 12.1% (by decarboxylation), 12.7% (by carbazole estimation).

The swollen gum, remaining after centrifugation, was stirred with water (3 l.) at 65-70° for twenty hours, dilute ammonia being added to maintain the solution in the pH range 6-8, thus preventing inadvertent autohydrolysis. Insoluble material was removed by centrifugation, and the supernatant liquid was treated as before to give polysaccharide II (1.0 g.), $[\alpha]_D = -54^\circ$ ($c = 1.0$); uronic acid anhydride content = 11.5% (by decarboxylation), 10.9% (by carbazole estimation).

The remaining swollen material was stirred in 5% ammonia

at 65-70° for twenty hours. This treatment was found adequate to dissolve all remaining swollen gum, leaving only pieces of wood or bark, in suspension. The supernatant liquid after filtration was treated as above to give polysaccharide III (1.7 g.), $[\alpha]_D = -58°$ ($c = 0.86$); uronic acid anhydride content = 11.6% (by decarboxylation), 11.6% (by carbazole estimation).

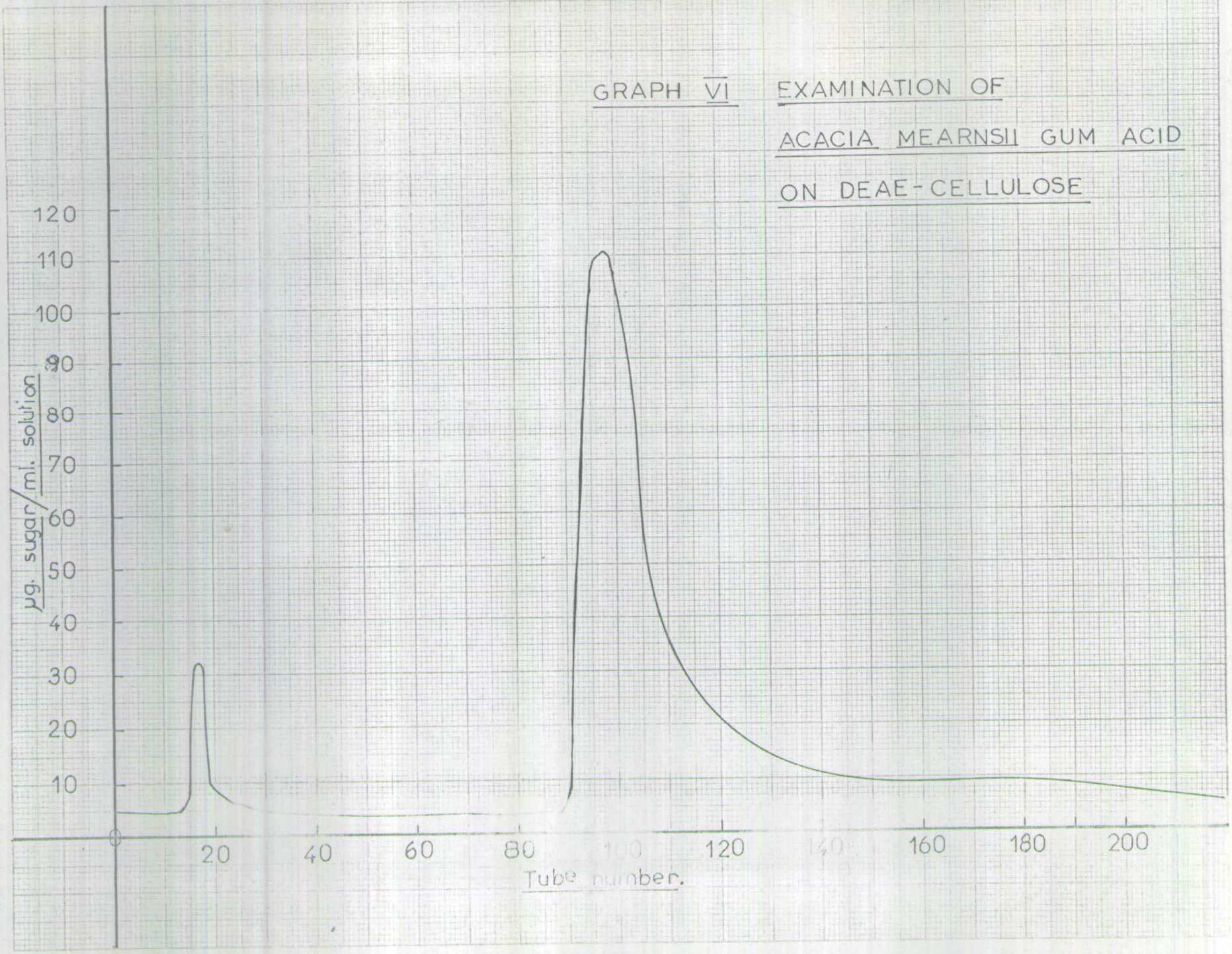
Polysaccharides I, II and III were kept separate and examined by small scale hydrolysis.

A sample of each polysaccharide fraction (3-5 mg.) was hydrolysed by N-sulphuric acid (1-2 ml.) at 100° for six hours in a sealed tube. After neutralisation and removal of cations the hydrolysates were examined by paper chromatography in solvents A, B and C. All three hydrolysates contained galactose, arabinose, rhamnose and acidic components, in identical relative proportions (visual examination of paper chromatogram).

Assessment of homogeneity

The gum acid (0.05 g.) in water (5 ml.) was stirred with Amberlite resin IR-120(H^+), in order to ensure the complete removal of metal ions. After filtration the solution was pipetted on to the top of a DEAE-cellulose column (4.2 x 30 cm.; 30 g. dry powder) which had been prepared in the usual way. The polysaccharide was allowed to remain on the column for twenty-four hours, before successive elution as follows:

GRAPH VI EXAMINATION OF ACACIA MEARNSII GUM ACID ON DEAE-CELLULOSE



a) 0.05 M (500 ml.) } of sodium dihydrogen
b) 0.10 M (500 ml.) } phosphate buffered
c) 0.25 M (500 ml.) } at pH 6 with sodium
d) 0.50 M (500 ml.) } hydroxide

e) 0.50 M (1000 ml.) potassium chloride solution

The flow rate of the column was adjusted to 25 ml. per hour, fractions being collected every half hour. The amount of polysaccharide present in each tube was measured by the phenol-sulphuric acid method, using a standard calibration curve based on D-galactose. A graph of polysaccharide content against tube number and hence strength of buffer is shown on Graph VI. Two peaks were obtained which accounted for approximately 4% and 80% of the polysaccharide material originally applied to the column.

Fraction 1 (tubes 15-18). This, the minor fraction, which was eluted by 0.05 M phosphate buffer had a uronic acid anhydride content of 0.2% (estimated by the carbazole method). An examination of this fraction by paper chromatography revealed the presence of the monosaccharides galactose and arabinose. Further examination of this fraction was not carried out.

Fraction 2 (tubes 90-105). This, the major fraction, contained approximately 80% of the original polysaccharide. It was eluted by 0.25 M phosphate buffer and had a uronic acid anhydride content of 13.1% (carbazole estimation).

Isolation and quantitative estimation of the component sugars of the gum

a) Mild partial hydrolysis

Purified gum acid (0.05 g.) was hydrolysed with 0.1 N-sulphuric acid (5 ml.) at 100° for one hour. The hydrolysate after neutralisation and removal of cations was poured into ethanol (50 ml.). The precipitated polysaccharide after re-precipitation was dissolved in water (10 ml.) and freeze-dried to give the degraded polysaccharide (0.024 g.). Hydrolysis of the degraded polysaccharide with N-sulphuric acid at 100° (4 hr.) gave galactose, acidic sugars, and a slight trace of arabinose, but no rhamnose.

The ethanolic centrifugates were combined and concentrated to give a dried syrup (0.024 g.) which when examined in solvents A and B gave evidence for a large amount of arabinose, a small amount of rhamnose and a trace quantity of galactose.

These sugars were separated by thick paper chromatography in solvent A. All components other than arabinose and rhamnose were discarded. The amounts obtained were,

L-arabinose	13.5 mg.
L-rhamnose	3.0 mg.

Estimation of these amounts was by the phenol-sulphuric acid method using calibration curves appropriate to each sugar. The above amounts give a molar ratio of almost 5:1 for the arabinose : rhamnose ratio.

b) Total hydrolysis

Gum acid (0.08 g.) was hydrolysed with N-sulphuric acid (10 ml.) at 100° (5 hr.). After cooling D-xylose (0.015 g.) was added as internal standard.

After neutralisation and removal of cations the hydrolysate was concentrated and examined by paper chromatography in solvents A and B. This indicated the presence of galactose, arabinose, xylose, rhamnose and acidic sugars. This mixture was separated by thick paper chromatography in solvent A. The absolute amount of each sugar was estimated by the phenol-sulphuric acid method using standard curves appropriate to each sugar. In the case of the acidic sugars, estimation was made with reference to a curve lying mid-way between that for galactose and that for glucuronic acid (on the assumption that the absorption due to glucuronic acid and 4-O-methyl glucuronic acid were identical). The estimated amounts were then corrected for loss during neutralisation, with reference to the amount of xylose recovered. The results are given below (Table XI).

Table XI

Sugar	Amount isolated	Galactose correction (a)	Rhamnose correction (b)	Milli-moles x 10	Molar ratio
D-galactose	28.9	30.9	30.9	1.72	4.4
L-arabinose	29.4	29.4	29.4	1.96	5.0
L-rhamnose	6.1	6.1	6.5	0.39	1.0
Acidic components	12.1	10.1	10.1	0.52	1.35

All weights are given in mg.

(a) Due to the fact that it has previously been established that the uronic acid content was 12.1%, i.e. 10.1 mg., the excess of this must be explained by the fact that galactose is still locked up as aldobiouronic acid - hence the galactose correction.

(b) The above mild hydrolysis designed so as to limit the degradation of rhamnose, showed that the arabinose to rhamnose ratio was 5:1. Since the ratio of amounts isolated in the more drastic hydrolysis is greater than 5:1 suggested that a preferential degradation of rhamnose had taken place, the amount of rhamnose isolated was corrected by an amount sufficient to maintain the 5:1 ratio - hence the rhamnose correction.

Examination of the gum for arabinobioses produced on partial hydrolysis

A sample of gum acid (0.20 g.) in water (20 ml.) was heated at 100°. Aliquot samples (4 ml.) were removed every four hours over a period of two days. Each was poured into ethanol (20 ml.) and any degraded gum removed at the centrifuge.

The centrifugates were concentrated to syrups which were examined chromatographically in solvents A, B and D.

Evidence was obtained for the presence of 3-O-β-L-arabinopyranosyl-L-arabinose, but no indication of any other arabinobiose was observed.

Partial acid hydrolysis of the gum acid and characterisation of the acidic fragments

Gum acid (3 g.) was hydrolysed by N-sulphuric acid (300 ml.) at 90-95° for six hours. After neutralisation and removal of cations, the hydrolysate was concentrated to a syrup (3.01 g.) which was adsorbed on a column of DEAE-Sephadex (12 g.; 2 x 15 cm.), prepared in the formate form.

Elution of the column with carbonate free distilled water (2 l.) and concentration of the eluate gave a syrup (2.15 g.) which partly crystallised. Chromatographic examination of the syrup in solvents A, B and D showed the presence of galactose, arabinose, rhamnose and traces of compounds having identical chromatographic mobility to 1→3 and 1→6 linked galactobioses. This neutral fraction was not examined further.

Elution of the column with 3% formic acid (3 l.) until the eluate was no longer positive towards the phenol-sulphuric acid reagent, removed the acidic sugars. The eluate was concentrated to small bulk (50 ml.) and extracted with ether until substantially free of formic acid (smell). Concentration of the aqueous layer gave a syrup (0.65 g.) which on chromatographic examination in solvents B and C established that, as a main component, it contained 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, which was accompanied by a number of other sugars.

Chromatographic separation of the mixture of sugars on Whatman No. 31 filter sheets using solvent B gave the three components of highest chromatographic mobility as pure

compounds, and a mixture of sugars which was refractionated to give three further components.

Identification of the fractions

Fraction 1. (0.02 g.); R_{gal} = 1.54 in solvent B; $[\alpha]_D = +49^\circ$ (c = 1.0).

Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave a sugar which was chromatographically indistinguishable from 4-O-methyl-D-glucose. This fraction was thus suspected to contain 4-O-methyl-D-glucuronic acid and so was not examined further.

Fraction 2. (0.050 g.); R_{gal} = 1.05 in solvent B; $[\alpha]_D = +40^\circ$ (c = 1.0).

This fraction was chromatographically homogeneous and indistinguishable from D-glucuronic acid. Reduction of the derived methyl ester methyl glycosides followed by hydrolysis gave glucose as the only sugar. This fraction was thus shown to contain D-glucuronic acid and so further examination was not attempted.

Fraction 3. (0.102 g.); R_{gal} = 0.49 in solvent B; $[\alpha]_D = +1^\circ$ (c = 1.0).

On paper chromatographic examination in solvents B and C this sugar was homogeneous and indistinguishable from 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. On hydrolysis and subsequent paper chromatographic examination of the hydrolysate in solvent B, both 4-O-methyl-glucuronic acid and galactose were detected on spraying with aniline oxalate.

Reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave 4-O-methyl glucose and galactose. A small scale methylation of the sample by the Kuhn procedure was carried out. After methanolysis of the methylated product and gas-liquid chromatography of the methyl glycosides, evidence was obtained for the presence of the following sugars,

Methyl glycoside of:	Retention times (T) Column C
2,3,4-tri-O-methyl-D-glucuronic acid	3.16, 2.36
2,3,5-tri-O-methyl-D-galactose	4.16
2,3,4-tri-O-methyl-D-galactose	7.00

Fraction 4. (0.224 g.); R_{gal} = 0.15 in solvent B; $[\alpha]_D = -7.5^\circ$ (c = 1.1).

This sugar was chromatographically homogeneous and indistinguishable from authentic 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. Hydrolysis gave glucuronic acid and galactose in approximately equal proportions (visual examination of paper chromatogram). Reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave glucose and galactose. This oligosaccharide was thus identical to that sugar previously characterised as 6-O-(β-D-glucopyranosyluronic acid)-D-galactose[50].

Fraction 5. (0.010 g.); R_{gal} = 0.09 in solvent B; $[\alpha]_D = +8^\circ$ (c = 1.0).

Hydrolysis of the sugar gave galactose, 4-O-methyl-D-glucuronic acid and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. Reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave 4-O-methyl glucose and galactose. A mild hydrolysis of the reduced sugar with 0.5 N-sulphuric acid at 100° for one hour gave a number of products, among which 6-O-β-D-galactosyl-D-galactose was recognized on account of its chromatographic mobility. Determination of the uronic acid anhydride content by the carbazole method, combined with total sugar content by the phenol-sulphuric acid method, showed that the ratio of acid to total sugar content was 1:2.8.

Fraction 6. (0.024 g.); R_{gal} = 0.04 in solvent B; $[\alpha]_D = +25^\circ$ (c = 1.2).

This component was chromatographically pure and from its mobility had the appearance of a trisaccharide. Hydrolysis gave galactose, glucuronic acid and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. Reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and glucose. A partial hydrolysis of the reduced oligosaccharide with 0.5 N-sulphuric acid at 100° for one hour gave a number of products, among which 6-O-β-galactosyl-galactose was recognized. A determination of the uronic acid content, as in fraction 5, gave the ratio of acid to total sugar as 1:2.8.

A small sample (5 mg.) was methylated by six additions of methyl sulphate (0.5 ml.) and sodium hydroxide (1 ml.). After

completion of the Haworth methylation in the normal way, the product was methanolised and the methyl glycosides examined by gas-liquid chromatography. The presence of the following methyl glycosides was indicated:

Methyl glycoside of:	Retention times (T) Column C
2,3,4-tri-O-methyl-D-glucuronic acid	3.16, 2.44
2,3,4-tri-O-methyl-D-galactose	7.05
2,3,5-tri-O-methyl-D-galactose	4.13
Unknown sugar	5.20

Smith degradation of degraded gum A

Previous experiments[50] showed that autohydrolysis of the gum, followed by a change in the specific rotation of the solution ($[\alpha]_D = -61^\circ \rightarrow +30^\circ$), effected complete removal of arabinose. This scheme was adopted in the formation of the degraded gum A used below. A sample of this degraded gum A was methylated in the same manner as previously[50]. The methylated polysaccharide (OMe, 43.4%, $[\alpha]_D = -41^\circ$, 0.92 in $CHCl_3$) was isolated in the usual manner, and a sample methanolysed by 4% methanolic hydrochloric acid for eighteen hours. Examination of the methyl glycosides by gas-liquid chromatography indicated the presence of the same methylated sugars in the same relative proportions as was found previously[50]. This sample of degraded gum A was thus identical to that isolated after the previous autohydrolysis.

The degraded gum acid (1 g., $[\alpha]_D = 0^\circ$ (c = 1.0), uronic acid anhydride content = 19%) was oxidised by sodium periodate (4.5 g.) in water (100 ml.) at room temperature in the dark. The consumption of oxidant was measured spectrophotometrically[81]. Over the first eight hours, approximately 3 moles of oxidant were consumed for every 2 moles of sugar residue, and no further uptake of oxidant was observed over the next twenty-four hours. The reaction was stopped after two days by the addition of ethylene glycol (1.34 g.), calculated to be only slightly in excess of the required amount for total destruction of all remaining periodate. The removal of cations was effected by treatment with Amberlite resin IR-120(H^+); the iodate was removed on treatment with barium carbonate. Insoluble salts were removed at the centrifuge and washed with water (4 x 50 ml.). The centrifugate and washings were concentrated (50 ml.) and potassium borohydride (1 g.) was added to the reaction mixture which was allowed to stand for two days. Removal of potassium ions was then effected on treatment with cationic exchange resin (in a column, 1 x 20 cm.). The de-ionised solution (500 ml.) was evaporated to almost dryness, and boric acid removed as methyl borate by successive evaporations with methanol (8 x 25 ml.).

The polyalcohol so formed (0.410 g.) was taken up in water (60 ml.) and 4 N-sulphuric acid (20 ml.) added. The hydrolysis was allowed to proceed at room temperature for twelve hours when it was stopped by neutralisation in the usual way. Removal of cations and concentration gave a thin syrup (ca. 2 ml.) which

when poured into ethanol (20 ml.) produced a white precipitate which after further washing in ethanol (5 x 50 ml.) was pistol dried to yield degraded gum B, (0.075 g.); $[\alpha]_D = 0^o$ (c = 1.01).

The ethanolic washings were combined and concentrated to give a thin dried syrup (0.257 g.), which failed to yield any monosaccharides after hydrolysis.

A small sample (10 mg.) of degraded gum B was hydrolysed with 0.5 N-sulphuric acid for one hour at 100°. After neutralisation, removal of cations and concentration, the syrup was examined chromatographically in solvents A, B and D. This indicated the presence of galactose, 3-O-β-galactosyl-galactose (R_{gal} = 0.48) and O-β-galactosyl-(1→3)-O-β-galactosyl-(1→3)-galactose (R_{gal} = 0.17) and higher homologues. It was noticeable that 6-O-β-galactosyl-galactose (R_{gal} = 0.33) was not detected on the paper chromatogram.

Degraded gum B was examined chromatographically in solvent H, and was found to be immobile.

Methylation of degraded gum B

The methylation of degraded gum B was carried out in three stages.

(a) The polysaccharide (0.04 g.) was dissolved in water (5 ml.), and methyl sulphate (1 ml.) and 30% sodium hydroxide (2 ml.) were added together over a period of two hours. Further six additions of methyl sulphate (2.5 ml.) and 30% sodium hydroxide (5 ml.) were added over five hours, one

addition being made each day for six days. During these additions the reaction mixture was kept in an atmosphere of nitrogen. One day after the final addition the flask and contents were heated on a boiling water bath for thirty minutes to destroy all remaining methyl sulphate. The pH of the solution was adjusted to 4 by the addition of 2 N-sulphuric acid. The resulting solution was extracted with chloroform (5 x 150 ml.) and the dried chloroform extracts concentrated to a syrup (0.022 g.).

(b) The partially methylated product, from (a), was dissolved in tetrahydrofuran (5 ml.), and dry powdered sodium hydroxide (5 g.) and methyl sulphate (5 ml.) were added in portions over four days, during which time the flask was continually shaken. After two days, additional tetrahydrofuran had to be added in order to maintain the mobility of the reaction mixture. After all reagents had been added, the reaction mixture was diluted by the addition of water (10 ml.). Again after heating at 100° to destroy all unreacted methyl sulphate, the solution was acidified (pH 4) with 2 N-sulphuric acid, and extracted with chloroform (5 x 150 ml.). The dried chloroform extracts were concentrated to a syrup (0.019 g.), (found: OMe, 37.8%).

(c) The partially methylated product from (b) was refluxed in iodomethane (4 ml.) and silver oxide (0.02 g.) was added in portions over four hours. The mixture was stirred under reflux for twenty hours after which the methylated product was extracted into boiling chloroform. The dried

chloroform extracts were concentrated to give a syrup (found: OMe, 39.0%). After four additional Purdie methylations, using amounts of reagents as above, the methylated degraded gum B (0.01 g.) was isolated from chloroform solution and had $[\alpha]_D = -6^\circ$ (c = 1.0 in $CHCl_3$), (found: OMe, 42.5% - not raised after a further methylation).

A sample of fully methylated degraded gum B (6 mg.) was methanolysed, and the methyl glycosides divided into two portions.

(i) Hydrolysis of the methyl glycosides (3 mg.) with N-sulphuric acid at 100° for four hours, gave the corresponding methylated monosaccharides. Chromatographic examination of these in solvents E and F, in comparison with authentic standards showed the presence of 2,3,4,6-tetra-, and 2,4,6-tri-O-methyl-D-galactose as main components. There was, however, in addition to these, a trace of 2,6-di-O-methyl-D-galactose.

(ii) The remainder of the methyl glycosides (3 mg.) were examined by gas-liquid chromatography. Methyl glycosides of the following sugars were detected:

Methyl glycoside of:	Retention times (T)	
	Column b	Column c
2,3,4,6-tetra-O-methyl-D-galactose	1.64	1.80
2,4,6-tri-O-methyl-D-galactose	2.38, 2.08	4.28, 3.67

ACKNOWLEDGEMENTS

The author wishes to thank Professor Sir Edmund Hirst, C.B.E., F.R.S., for the provision of laboratory facilities, Dr. G.O. Aspinall for his valuable advice and encouragement during the course of this research, and the Science Research Council for the award of a research studentship.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. R.L. Whistler and J.N. BeMillar, Advances in Carbohydrate Chemistry, 1958, 13, 289.

2. H. Neukom and H. Deuel, Chem. and Ind., 1958, 36, 683.

3. P. Heim and H. Neukom, Helv. Chim. Acta, 1962, 45, 1737.

4. G.O. Aspinall and V.P. Bhavanandan, J. Chem. Soc., 1965, 2685, 2693.

5. G.O. Aspinall, M.J. Johnston and A.M. Stephen, J. Chem. Soc., 1960, 4918.

6. G.O. Aspinall, E.L. Hirst and N.K. Matheson, J. Chem. Soc., 1956, 989.

7. F. Smith and R. Montgomery, "The Chemistry of Plant Gums and Mucilages." Reinhold Publishing Corporation, New York, 1959.

8. S.P. James and F. Smith, J. Chem. Soc., 1945, 739, 746, 749.

9. G.O. Aspinall and J. Baillie, J. Chem. Soc., 1963, 1702, 1714.

10. J.M. McNab, Ph.D. Thesis, Edin. Univ., 1965.

11. H. El-Khadem and M.M. Megahed, J. Chem. Soc., 1956, 3953.

12. J.E. Scott, "Methods in Carbohydrate Chemistry," V, p. 38.

13. E. Hägglund, B. Lindberg and J. McPherson, Acta Chem. Scand., 1956, 10, 1160.

14. W. Heri, H. Neukom and H. Deuel, Helv. Chim. Acta, 1961, 44, 1939.

15. T. Svedberg and K.O. Pederson, "The Ultracentrifuge," Oxford Univ. Press, 1940.

16. W.W. Binkley, Advances in Carbohydrate Chemistry, 1955, 10, 55.

17. M. Dubois, K.A. Gillies, J.K. Hamilton, P.A. Rebers and F. Smith, Analyt. Chem., 1956, 28, 350.

18. D.M.W. Anderson, Talanta, 1959, 2, 73.

19. G.O. Aspinall, A.J. Charlson, E.L. Hirst and R. Young, J. Chem. Soc., 1963, 1696.

G.O. Aspinall and R. Young, J. Chem. Soc., 1965, 3003.

20. G.O. Aspinall, I.M. Cairncross and A. Nicolson, Proc. Chem. Soc., 1959, 270.

21. A.M. Unrau, Chem. and Ind., 1960, 1419.

I.J. Goldstein, G.W. Hay, B.A. Lewis and F. Smith, Abs. of 135th Amer. Chem. Soc. meeting, Boston, Mass., April 1959, p. 3D.

22. F. Smith and D.F. Spriestersbach, Abs. of 128th Amer. Chem. Soc. meeting, Minneapolis, Minn., 1955, p. 15D, quoted in ref. 7, page 255.

23. C.L. Butler and L.H. Cretcher, J. Amer. Chem. Soc., 1930, 52, 4509.

24. R.D. Hotchkiss and W.F. Goebel, J. Amer. Chem. Soc., 1936, 58, 855.

25. F. Smith, J. Chem. Soc., 1939, 744.

26. J. Jackson and F. Smith, J. Chem. Soc., 1940, 79.

27. G.O. Aspinall, Chim. Biochim. Lignine Cellulose Hemicelluloses, Actes Symp. Intern., p. 421, Les Imprimeries Réunies de Chambéry, France (1964).

28. G.O. Aspinall and K.M. Ross, J. Chem. Soc., 1963, 1681.

29. G.O. Aspinall and J.N.C. Whyte, J. Chem. Soc., 1964, 5058.

30. H. Meier, Acta Chem. Scand., 1960, 14, 749.

31. R.M. Fairweather, Ph.D. Thesis, Edin. Univ., 1965.

32. V.M. Parikh and J.K.N. Jones, "Proceedings of Fifth Cellulose Conference." J. Polymer Science, 1965, 11, 139.

33. G.O. Aspinall, M.J. Johnston and R. Young, J. Chem. Soc., 1965, 2701.

34. G.O. Aspinall, R.N. Fraser and G.R. Sanderson, J. Chem. Soc., 1965, 4325.

35. G.O. Aspinall, E.L. Hirst and A. Nicolson, J. Chem. Soc., 1959, 1697.

36. G.O. Aspinall and R.M. Fairweather, Carbohydrate Res., 1965, 1, 83.

37. H.O. Bouveng and B. Lindberg, Acta Chem. Scand., 1958, 12, 1977.

38. H.O. Bouveng, Acta Chem. Scand., 1959, 13, 1869.

39. J.K.N.Jones, J. Chem. Soc., 1953, 1672.

40. H.O. Bouveng, Acta Chem. Scand., 1959, 13, 1877.

41. H.O. Bouveng, Acta Chem. Scand., 1961, 15, 78.

42. G.O. Aspinall and T.M. Wood, J. Chem. Soc., 1963, 1686.

43. B. Urbas, C.T. Bishop and G.A. Adams, Canad. J. Chem., 1963, 41, 1522.

44. J.K.N. Jones and P.E. Reid, J. Polymer Science, 1963, No. C, vol. II, 63.

45. A.J. Roudier and L. Eberhard, Bull. Soc. Chim., 1963, 844.

46. G.O. Aspinall and R.S. Fanshawe, J. Chem. Soc., 1961, 4215.

47. I.M. Morrison, Ph.D. Thesis, Edin. Univ., 1965.

48. G.O. Aspinall and J.W.T. Craig, Unpublished results.

49. G.O. Aspinall and R.N. Fraser, J. Chem. Soc., 1965, 4318.

50. G.O. Aspinall, J.J. Carlyle and R. Young, Unpublished results, quoted in R. Young, Ph.D. Thesis, Edin. Univ., 1963.

51. J.K.N. Jones and G.H.S. Thomas, Canad. J. Chem., 1961, 39, 192.

52. A.K. Bhatacharyya and C.V.N. Rao, Canad. J. Chem., 1964, 42, 107.

A.K. Bhatacharyya and A.K. Mukerjee, Bull. Chem. Soc. Japan, 1964, 37, 1425.

53. A.M. Stephen, J. Chem. Soc., 1957, 1919.

54. F. Smith and A.M. Stephen, J. Chem. Soc., 1961, 4892.

55. A.M. Stephen, J. Chem. Soc., 1962, 2030.

56. A.M. Stephen, J. Chem. Soc., 1963, 1974.

57. A.M. Stephen, South African Industrial Chemist, 1963, 83.

58. V. Zitco, J. Rosik, M. Brutenicova and J. Kubala, Coll. Czechoslovak. Chem. Commun., 1965, 30, 3501.

59. G.O. Aspinall, E.L. Hirst and A. Wickstrøm, J. Chem. Soc., 1955, 1160.

60. G.O. Aspinall, B.J. Auret and E.L. Hirst, J. Chem. Soc., 1958, 221, 4408.

61. G.O. Aspinall and T.B. Christensen, J. Chem. Soc., 1965, 2673.

62. G.O. Aspinall, V.P. Bhavanandan and T.B. Christensen, J. Chem. Soc., 1965, 2677.

63. G.O. Aspinall and T.B. Christensen, J. Chem. Soc., 1961, 3461.

64. G.O. Aspinall and J.M. McNab, Chem. Comm., 1965, 565.

65. G.O. Aspinall and A. Rudowski, Unpublished results.

66. L. Hough, J.K.N. Jones and W.H. Wadman, J. Chem. Soc., 1949, 2511.

67. W.E. Trevelyan, D.P. Procter and J.S. Harrison, Nature, 1950, 166, 444.

68. R.U. Lemieux and H.F. Bauer, Analyt. Chem., 1954, 26, 920.

69. F.E. Hardy and J.G. Buchanan, J. Chem. Soc., 1963, 5881.

70. R. Consden and W.M. Stanier, Nature, 1952, 169, 783.

71. R.L. Whistler and D.F. Durso, J. Amer. Chem. Soc., 1950, 72, 677.

72. R. Kuhn, H. Trischman and I. Löw, Angew. Chem., 1955, 62, 32.

73. G.O. Aspinall, J. Chem. Soc., 1963, 1676.

74. C.T. Bishop and F.P. Cooper, Canad. J. Chem., 1960, 38, 388.

75. S. Allen, T.G. Bonner, E.J. Bourne and N.M. Saville, Chem. and Ind., 1955, 33, 1449.

76. L. Hough, J.K.N. Jones and W.H. Wadman, J. Chem. Soc., 1950, 1702.

77. W.J. Whelan and K. Morgan, Chem. and Ind., 1955, 33, 1449.

78. R. Belcher and A.L. Godbert, "Semi-micro Quantitative Organic Analysis," Longmans, Green and Co., 1954.

79. R.U. Lemieux and H.F. Bauer, Canad. J. Chem., 1953, 31, 814.

80. E.A. McComb and R.M. McCready, Analyt. Chem., 1952, 24, 1630.

81. G.O. Aspinall and R.J. Ferrier, Chem. and Ind., 1957, 1216.

82. C.T. Bishop, Canad. J. Chem., 1955, 33, 1521.

83. G.O. Aspinall and M. Uddin, Unpublished results.

84. A.M. Stephen, J. Chem. Soc., 1951, 646.

85. A.M. Stephen, Private communication.

86. A.J. Charlson, J.R. Nunn and A.M. Stephen, J. Chem. Soc., 1955, 1428.

87. B. Wickberg, Acta Chem. Scand., 1958, 12, 615.

88. F. Brown, E.L. Hirst and J.K.N. Jones, J. Chem. Soc., 1949, 1757.

89. P. Andrews, L. Hough and D.B. Powell, Chem. and Ind., 1956, 658.

90. C.N. Turton, A. Bebbington, S. Dixon and E. Pacsu, J. Amer. Chem. Soc., 1955, 77, 2565.

91. D.A. Rees and J.W.B. Samuel, Chem. and Ind., 1965, 2008.

92. D.A. McFadyen, J. Biol. Chem., 1945, 158, 107.

93. J.K.N. Jones, R.A. Wall and A.O. Pittet, Canad. J. Chem., 1960, 38, 2285.

94. A.J. Charlson, P.A.J. Gorin and A.S. Perlin, Canad. J. Chem., 1956, 34, 1811.

95. S. Peat, W.J. Whelan and T.E. Edwards, J. Chem. Soc., 1961, 29.

96. S. Peat, J.R. Turvey and D. Doyle, J. Chem. Soc., 1961, 3918.

97. Y.C. Lee and C.E. Ballou, Biochemistry, 1965, 4, 257.

ABSTRACT OF THESIS

Name of Candidate JOHN JARDINE CARLYLE

Address Esk Dene, Lockerbie, Dumfriesshire

Degree Doctor of Philosophy *Date* September, 1966

Title of Thesis The molecular structure of exudate gums, with special reference to gums of the Combretaceae

---

Anogeissus leiocarpus gum has been fractionated to give two polysaccharide fractions, leiocarpan A and leiocarpan B.

Studies on leiocarpan A have been directed towards an understandin of the peripheral structure of the molecule. In addition to monosaccharides the following oligosaccharides were liberated by mild hydrolysis and characterised:

3-O-β-L-arabinofuranosyl-L-arabinose
3-O-β-L-arabinopyranosyl-L-arabinose
3-O-β-D-galactopyranosyl-D-galactose
6-O-β-D-galactopyranosyl-D-galactose
O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-D-galactose
3-O-β-D-galactopyranosyl-L-arabinose
O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-L-arabinose
2-O-(β-D-glucopyranosyluronic acid)-D-mannose
6-O-(β-D-glucopyranosyluronic acid)-D-galactose
O-(β-D-glucopyranosyluronic acid)-(1→6)-D-galactose-(1→3)-L-arabinose

Hydrolysis of reduced methylated leiocarpan A gave

2,3,5- and 2,3,4-tri-, 2,5-, 2,3-, 3,5- and 2,4-di-, and 2-mono-O-methyl-L-arabinose, 2,3,4,6-tetra, 2,4,6-tri-, 2,4-di- and 2-mono-O-methyl-D-galactose, 3,4,6-tri-, 3,4-di- and 4-mono-O-methyl-D-mannose, 2,3-di-O-methyl-D-glucose, and 2,3,4-tri-O-methyl-D-xylose together with other sugars.

The stepwise degradation of carboxyl reduced leiocarpan A by the Smith procedure gave a series of degraded gums each of which contained galactose, arabinose and mannose, the probable sequence of the residues in these degraded gums being shown by partial hydrolysis and methylatio studies.

The structural features of leiocarpan A are discussed on the basis of these and previous results.

Leiocarpan B was examined by partial hydrolysis and methylation analysis. From these results the probable structural features of leiocarpan B are discussed in the light of those of leiocarpan A.

A /

Use other side if necessary.

A re-examination of the acidic oligosaccharides from the partial hydrolysis of gum ghatti has shown that a component previously thought to be a trisaccharide is O-(β-D-glucopyranosyluronic acid)-(1→2)-O-α-D-mannosyl-(1→4)-O-(β-D-glucopyranosyluronic acid)-(1→2)-D-mannose, which has also been isolated from leiocarpan A.

Acacia mearnsii gum was degraded by the Smith procedure to give a degraded gum, which by partial hydrolysis and methylation analysis was shown to be composed mainly, if not entirely, of 1→3 β-linked D-galactopyranose residues.

On the basis of these and previous results the probable structural features of A. mearnsii gum are discussed and compared with those of gum arabic and other Acacia gums.